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Pain in Multiple Sclerosis

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Contents

Abbreviations	xxix
Acknowledgements.....	xxxv
Declaration.....	xxxvii
Abstract.....	xxxix
Lay Summary	xlili
Chapter 1 Introduction	1
1.1 Background	1
1.1.1 Key definitions: pain	1
Definition of pain	1
Definition of neuropathic pain in this thesis	1
Definition of neuropathic limb pain in this thesis.....	1
1.1.2 Why is pain in multiple sclerosis an important research target?.....	2
1.1.3 Epidemiology of pain in multiple sclerosis.....	8
1.1.4 The descending pain modulatory system (DPMS)	9
Overview of DPMS.....	9
Is there evidence consistent with DPMS dysfunction in pain related to MS? 12	
1.1.5 Neuroimaging of pain in multiple sclerosis	25
Fundamentals of magnetic resonance imaging	25
Fundamentals of functional MRI imaging	26
Magnetic Resonance Imaging in multiple sclerosis.....	27
Structural MRI brain imaging of pain and related phenotypes.....	28
Structural MRI brain imaging of the MS disease process	28
1.1.6 Functional neuroimaging relevant to pain in multiple sclerosis	38
Resting state fMRI	38
Functional MRI in multiple sclerosis.....	40
Functional anatomy of descending pain modulatory system	42
Structural imaging of cervical cord.....	47
1.2 Overview of thesis structure.....	49
1.2.1 Systematic Reviews	49
1.2.2 Prospective clinical study.....	49

1.3	Aims of thesis.....	50
1.3.1	Systematic Review: Prevalence, associations and natural history of pain in multiple sclerosis.....	50
	Pain prevalence	50
	Pain incidence	50
	Natural history of pain in MS.....	50
	Relevance to design of clinical study	51
1.3.2	Systematic Review: Neuroimaging correlates of pain syndromes in multiple sclerosis.....	52
	Relevance to clinical study.....	52
1.3.3	Prospective experimental study: Clinical, behavioural and neuropsychological associations of neuropathic limb pain in adults with relapsing remitting multiple sclerosis	53
1.3.4	Prospective experimental study: Structural imaging associations of neuropathic limb pain in adults with relapsing remitting multiple sclerosis.....	54
	Quantification of structural brain imaging characteristics	54
	Exploration of methods:	54
1.3.5	Prospective experimental study: Functional connectivity of the descending pain modulatory system in adults with neuropathic limb pain associated with multiple sclerosis	55
1.4	Hypotheses	56
1.4.1	Systematic Review: Prevalence, associations and natural history of pain in multiple sclerosis.....	56
1.4.2	Systematic Review: Neuroimaging correlates of pain syndromes in multiple sclerosis.....	56
1.4.3	Prospective experimental study: Overall.....	57
1.4.4	Prospective experimental study: Clinical, behavioural and neuropsychological associations of neuropathic limb pain in adults with relapsing remitting multiple sclerosis	57
1.4.5	Prospective experimental study: Structural imaging associations of neuropathic limb pain in adults with relapsing remitting multiple sclerosis.....	58
	Tissue class volumes	58

T2 Hyperintense lesion volume and distribution	58
Grey matter volumes	59
Exploration of cross-sectional cervical cord area from MPRAGE brain data	59
1.4.6 Prospective experimental study: Functional connectivity of the descending pain modulatory system in adults with neuropathic limb pain associated with multiple sclerosis	60
Chapter 2 Prevalence, Incidence and Clinical Associations of Pain in Adults with Multiple Sclerosis: Systematic Review and Meta-Analysis	61
2.1 Introduction	61
2.1.1 Peer-reviewed publication related to this work.....	62
2.2 Methods.....	63
2.2.1 Literature search and selection criteria	63
Search Strategies	63
Inclusion and exclusion criteria	65
Process of review by researchers	65
2.2.2 Data Extraction and Analysis.....	66
Data extraction	66
Quality Assessment of included studies.....	67
Meta-analytical and statistical analysis	67
2.3 Results	68
2.3.1 Characteristics and quality assessment of included studies	71
2.3.2 Prevalence of pain overall.....	75
2.3.3 Prevalence of specific pain syndromes	76
Nociceptive and Neuropathic pain	78
Pain at more than one site	79
Comparison of pain prevalence in MS subtypes.....	80
2.3.4 Associations of study-level pain prevalence: meta-regression analysis	81
2.3.5 Pain incidence	84
2.3.6 Pain prevalence at MS disease course milestones	84
Pain prevalence prior to disease onset	84
Pain prevalence at disease onset	84
Pain prevalence at relapse	84

Pain prevalence during disease evolution	85
2.3.7 Further literature since original searches, identified by cited reference search	85
Estimates of pain prevalence.....	85
Pain in MS relapse.....	86
Longitudinal characterisation and incidence of pain in MS.....	87
2.4 Discussion	90
Chapter 3 Neuroimaging of Pain Syndromes in Adults with Multiple Sclerosis: Systematic Review	91
3.1 Introduction	91
3.1.1 Peer-reviewed publication related to this work.....	91
3.1.2 Contributions.....	91
3.2 Materials and Methods	92
3.2.1 Literature search and selection criteria.....	92
Search strategies.....	92
Inclusion and Exclusion Criteria	94
Process of review by researchers	94
3.2.2 Data Extraction and Analysis.....	95
Data Extraction.....	95
Quality assessment of included studies	95
Categorisation of studies	96
Statistical analysis	96
3.3 Results	97
3.3.1 Design of experimental studies	99
3.3.2 Quality Assessment of included studies.....	104
Field Strength	107
MRI protocols	107
3.3.3 Diagnosis of multiple sclerosis in included studies	109
Subtype of MS.....	109
3.3.4 Pain syndromes assessed in included studies	110
3.3.5 Imaging correlates of pain syndromes in included studies	112
Measures of volume (brain or spinal cord)	112

Lesion location	112
3.3.6 Treatment of pain	115
3.3.7 Further literature identified by search update	115
Lincoln and colleagues, 2016 (212)	116
Seixas and colleagues, 2016 (214)	116
3.4 Discussion	118
Chapter 4 Clinical, behavioural and cognitive associations of neuropathic limb pain in adults with multiple sclerosis.....	119
4.1 Introduction	119
4.2 Methods.....	119
4.2.1 Ethics Committee Review.....	119
4.2.2 Participant Recruitment.....	119
Study groups: principal groups	119
Matching of groups	120
Participants with MS:	121
Healthy controls (for sensory testing only).....	123
4.2.3 Data acquisition.....	123
4.2.4 Study Measures	124
Demographics	124
Medication.....	124
Study Instruments: Pain	125
Study Instruments: Clinical assessment and symptoms.....	130
Study instruments: Neuropsychology	135
Study instruments: assessment of sensory symptoms.....	142
Sensory assessment	143
4.2.5 Analysis.....	144
Data anonymization	144
Data storage.....	144
Data marking and curation	144
4.2.6 Overall analysis strategy	145
Comparison of subjects with MS, with and without neuropathic limb pain	145
Correlates of pain severity in people with MS and neuropathic limb pain..	146

Type I error and correction for multiple comparisons	146
The role of medications	146
4.3 Results	147
4.3.1 Participant Recruitment	147
People with MS (with and without neuropathic pain)	147
4.3.2 Application of research definitions of neuropathic pain to study cohort	
149	
IASP criteria	149
EFNS criteria, 2010	149
4.3.3 Overview of participant medications	150
4.3.4 Associations of the presence of pain: comparison of MS subjects with	
and without neuropathic pain	158
Demographics and matching	158
Emotional and affective morbidity	160
Cognitive evaluation: Brief international Cognitive Assessment in MS	
(BICAMS)	162
Cognitive evaluation: executive function	163
Sensory evaluation: Quantitative Sensory Testing	165
Anatomical symptom distribution	169
Quality of life and sleep	171
4.3.5 Subgroup exploration of associations of the presence of pain: subjects	
not receiving adjuvant analgesics	173
Demographics and matching	173
Psychological and affective morbidity	174
Cognitive evaluation: Brief international Cognitive Assessment (BICAMS)	
.....	174
Cognitive evaluation: executive function	176
Sensory evaluation	178
4.3.6 Correlates of pain severity	179
Demographics	179
Pain severity measures	180
Psychological and affective morbidity	181

Cognitive evaluation: Brief International Cognitive Assessment in MS (BICAMS).....	182
Cognitive evaluation: executive function.....	183
Quality of life and related variables	184
4.3.7 Participant experience of study	185
4.4 . Discussion	186
Chapter 5 Structural imaging correlates of neuropathic limb pain in adults with multiple sclerosis.....	188
5.1 Introduction	188
5.2 Methods.....	189
5.2.1 Structural image acquisition.....	189
5.2.2 General Image Analysis	190
General preprocessing of imaging data.....	190
5.2.3 Reporting of statistical significance	191
Standard space templates	192
Anatomical localisation.....	192
Creation of three-dimensional masks for region of interest analyses.	193
5.2.4 Intracranial Volume Estimation: comparison of methods	195
General	195
Manual estimation of ICV:.....	195
Fully automated estimation of ICV	196
Comparison of manual and automated ICV estimation	197
5.2.5 Tissue type segmentation	198
General description and preprocessing	198
5.2.6 Lesion segmentation	198
Working definition of T2 hyperintense lesions.....	198
Independent blinded neuroradiologist review of imaging	198
Provisional automated lesion map generated for each subject.....	198
Creation of manually edited lesion map for each subject	199
Calculation of lesion volume in native space.....	201
Intra-rater reliability of manual lesion segmentation	201
5.2.7 Group-level lesion probability maps.....	202

Transforming single-subject lesion masks to standard space.....	202
Creating group-level lesion probability maps	202
5.2.8 Voxel Based Morphometry	203
Lesion filling	203
VBM processing.....	204
5.2.9 Exploratory analysis of cervical cord cross-sectional area from MPRAGE brain acquisitions.....	205
Review of available data	205
Image pre-processing	206
Manual estimation:.....	206
Automated estimation of CC-CSA:.....	207
Comparison of automated and manual estimation	210
Examining associations between cord cross-sectional area, imaging and clinical parameters.....	210
5.3 Results	211
5.3.1 Availability of data.....	211
5.3.2 Comparison of Intracranial Volume (ICV) estimation techniques	211
Visual comparison.....	211
Correlation of automated and semi-automated ICV estimates.....	211
5.3.3 Intra-rater reliability of manual white matter lesion segmentation	214
Correlation.....	214
Agreement between repeated measures	215
5.3.4 Associations of the presence of pain: comparison of MS subjects with and without neuropathic pain	216
Volumes of overall tissue classes.....	216
Lesion volume and distribution.....	217
Voxel Based Morphometry	224
5.3.5 Correlates of pain severity.....	228
Volumes of overall tissue classes.....	228
Lesion volume and distribution.....	228
Voxel Based Morphometry	230
5.3.6 Grey matter correlates of pain duration.....	231

Positive correlation of grey matter volume with pain duration.....	231
Negative correlation of grey matter volumes with pain duration	231
5.3.7 Exploratory analysis of cross-sectional cord area.....	232
Availability of data.....	232
Quality of data.....	232
Manual and automated cross-sectional area at P2.5 (spinomedullary junction)	
.....	232
Manual and automated cross-sectional area at C2/3 level	234
Use of automated estimates for further analysis	236
Correlation between CC-CSA estimates and ICV	237
Relationships between CC-CSA, ICV and clinical variables	238
Comparison of cord cross-sectional area in participants with and without pain	
.....	241
5.4 Discussion	242
Chapter 6 Functional connectivity of the descending pain modulatory system	
examined using resting state functional MRI.....	245
6.1 Introduction	245
6.2 Methods.....	246
6.2.1 Estimation of sample size	246
Existing studies: pain in pwMS examined using resting state fMRI	246
Existing studies: pain in healthy volunteers or subjects with other health	
conditions examined using resting state fMRI.....	247
Estimation for current study.....	247
6.2.2 Acquisition	248
Scanner	248
Field maps	248
Resting state functional data	248
Clinical data acquired in scanner	248
Instructions to participants	248
6.2.3 Preprocessing of resting state data	249
Preparation of fieldmap data	249
Preprocessing	249

Registration	250
Approach to potential sources of artefact and noise	250
6.2.4 Creation of region of interest masks	254
Rostral Anterior Cingulate Cortex (rACC) seed mask.....	254
Periaqueductal grey matter (PAG) region of interest mask	255
Bilateral dorsolateral prefrontal cortex (DLPFC) region of interest mask...	255
Occipital cortex region of interest “control” mask.....	256
Registration of rACC seed mask to resting state functional data.....	258
6.2.5 Analysis of fMRI data	258
Extraction of timecourses at subject level.....	258
Group level analyses	258
6.3 Results	261
6.3.1 Clinical data.....	261
6.3.2 Mean ACC seed connectivity by group	262
6.3.3 Associations of the presence of pain	263
Unpaired t-test: whole brain level	263
Unpaired t-test: PAG spherical ROI.....	263
Unpaired t-test: PAG spherical ROI after correction for T2 hyperintense lesion volume	265
Unpaired t-test: bilateral DLPFC region of interest	265
Unpaired t-test: bilateral DLPFC region of interest after correction for T2 hyperintense lesion volume	266
Unpaired t-test: “control” region of interest, occipital cortex	266
6.3.4 Associations of the severity of pain	267
Regression analysis: whole brain	267
Regression analysis: PAG region of interest.....	267
Regression analysis: DLPFC region of interest	267
Regression analysis: “control” region of interest, occipital cortex	268
6.3.5 Associations of T2 hyperintense lesion volume.....	269
6.4 Discussion	270
Chapter 7 Participant experience of the study	271
7.1 Introduction	271

7.1.1	Rationale for survey of participant experience	271
7.1.2	Aims	272
7.2	Methods.....	272
7.2.1	Ethical permissions	272
7.2.2	Identifying existing instruments.....	272
7.2.3	Survey design process	273
7.2.4	Survey design	273
7.2.5	Delivering the survey	273
7.2.6	Invitation to participate	274
7.2.7	Analysis.....	275
	Quantitative data	275
	Qualitative data	275
7.3	Results	276
7.3.1	Existing instruments.....	276
7.3.2	Participant input into survey design.....	276
7.3.3	Survey Uptake.....	276
7.3.4	Demographics	276
7.3.5	Comparison of survey responders and non-responders	276
7.3.6	Overall satisfaction with study participation	277
7.3.7	Information exchange and discussion	278
	Free text comments	278
7.3.8	Study procedures carried out at Rowling Clinic	279
	Free text comments	280
7.3.9	MRI imaging at Clinical Research Imaging Centre (CRIC).....	280
	Free text comments	281
7.3.10	fMRI procedures	282
	Free text comments	283
7.3.11	Motivation to participate in research.....	283
7.3.12	Changes suggested for future studies.....	285
7.3.13	Any specific strengths of study	286
7.3.14	Considering contribution to future study design.....	286
7.3.15	Considering participation in a future study	287

7.4	Discussion	288
Chapter 8	Discussion	289
8.1	Overview	289
8.1.1	Restatement of main study hypothesis	289
8.2	Findings	290
8.2.1	Systematic Review: Prevalence, associations and natural history of pain in multiple sclerosis.....	290
	Prevalence of pain, and pain syndromes	290
	Estimation of pain prevalence throughout the disease course.....	291
	Pain in MS relapse.....	292
	How does pain in MS vary with time?	292
8.2.2	Systematic Review: Neuroimaging correlates of pain syndromes in multiple sclerosis.....	293
	Study characteristics and methodology	293
8.2.3	Prospective experimental study: Clinical, behavioural and neuropsychological associations of neuropathic limb pain in adults with relapsing remitting multiple sclerosis	297
	Study groups: demographics and medication.....	297
	Differences between MS pain and MS control groups	297
	Correlates of pain severity.....	298
	Interpretation	299
8.2.4	Prospective experimental study: Structural imaging associations of neuropathic limb pain in adults with relapsing remitting multiple sclerosis....	300
	Comparison of groups with and without neuropathic pain	300
	Correlates of pain severity.....	301
	Correlates of pain duration.....	301
	Other findings.....	301
	Interpretation of findings.....	302
	Comments on exploration of cross-sectional cervical cord data, from available brain imaging acquisitions	306

8.2.5	Prospective experimental study: Functional connectivity of the descending pain modulatory system in adults with neuropathic limb pain associated with multiple sclerosis	310
	Mean functional connectivity of rACC seed in subjects with and without pain	310
	Lower connectivity between rACC seed and PAG, in pain group	310
	Higher connectivity between rACC seed and DLPFC, in pain group	310
	Adjusting for T2 hyperintense lesion volume diminishes or eliminates differential connectivity	311
	Increasing T2 hyperintense lesion volume is associated with differential rACC functional connectivity at the whole brain level.....	311
	Trends towards correlation of pain severity with rACC:DLPFC and rACC:PAG functional connectivity.....	311
	Anatomical specificity of differential connectivity.....	311
	Interpretation of findings.....	312
8.2.6	Findings of prospective study in relation to overarching study hypothesis	313
	Clinical and neuropsychological data	313
	Structural Imaging.....	313
	Functional imaging	314
8.2.7	Participant experience of study	314
	Overview	314
	Participation in feedback.....	314
	Overall satisfaction with study participation.....	315
	Experience of particular parts of the study	315
8.3	Relevance of this work to wider field	320
8.3.1	Systematic Review: Prevalence, associations and natural history of pain in multiple sclerosis	320
8.3.2	Systematic Review: Neuroimaging correlates of pain syndromes in multiple sclerosis.....	321
8.3.3	Clinical, behavioural and neuropsychological associations of neuropathic limb pain in adults with relapsing remitting multiple sclerosis ...	322

8.3.4	Structural imaging associations of neuropathic limb pain in adults with relapsing remitting multiple sclerosis	323
8.3.5	Functional connectivity of the descending pain modulatory system in adults with neuropathic limb pain associated with multiple sclerosis	324
8.4	Weaknesses of methodology	326
8.4.1	Systematic Review: Prevalence, associations and natural history of pain in multiple sclerosis.....	326
	Inconsistent methodology even in relatively homogeneous studies	326
8.4.2	Systematic Review: Neuroimaging correlates of pain syndromes in multiple sclerosis.....	327
	Definition of multiple sclerosis	327
	Limitation to English language studies	327
	Search strategies.....	328
	Quality assessment of studies.....	328
8.4.3	Prospective Study: general comments.....	328
	Cross-sectional study design	329
	Generalizability of data	329
8.4.4	Clinical, behavioural and neuropsychological associations of neuropathic limb pain in adults with relapsing remitting multiple sclerosis....	331
	Patient self-report measures	331
	Neuropsychology assessment.....	331
	Sensory testing	332
	Study analysis.....	332
	Multiple comparisons.....	332
	Effects of medication	333
8.4.5	Structural imaging associations of neuropathic limb pain in adults with relapsing remitting multiple sclerosis	339
8.4.6	Functional connectivity of the descending pain modulatory system in adults with neuropathic limb pain associated with multiple sclerosis	345
	Could these findings be an epiphenomenon related to altered grey matter volume?	345
	Could these findings be an artefact of CSF signal?	345

Could these findings reflect head motion during image acquisition?	346
Could these findings reflect differential lesion topography?	347
Could functional connectivity findings reflect differences in subjects' medications?.....	349
Use of seed based analysis, and default mode network (DMN)	352
8.4.7 Participant experience study	353
Timing of acquisition of data	353
Possible bias in participation.....	354
Sample size.....	354
Numerical outcomes.....	354
Questions used in instrument	354
General	355
8.5 Strengths of methodology	356
8.5.1 Systematic Review: Prevalence, associations and natural history of pain in multiple sclerosis	356
8.5.2 Systematic Review: Neuroimaging correlates of pain syndromes in multiple sclerosis.....	356
8.5.3 Clinical, behavioural and neuropsychological associations of neuropathic limb pain in adults with relapsing remitting multiple sclerosis ...	358
Clinical phenotyping	358
Burden to participants	358
Study assessments	358
8.5.4 Structural imaging associations of neuropathic limb pain in adults with relapsing remitting multiple sclerosis	359
Study design	359
Use of matched disease control group	359
High resolution isotropic structural imaging.....	359
Visual review of analysis data	360
8.5.5 Functional connectivity of the descending pain modulatory system in adults with neuropathic limb pain associated with multiple sclerosis	360
Clinical control group	360
Clinical phenotyping and structural imaging.	360

Previous studies.....	361
Processing strategies to minimise contribution of extraneous signal/noise .	361
Location and functional connectivity of seed and masks is comparable to previous literature.....	361
8.5.6 Participant Experience Study	362
Novelty of data	362
Pseudo-anonymisation of data	363
Participant engagement in development and execution of future studies at the Anne Rowling Regenerative Neurology Clinic	363
8.6 How has the field changed during the course of this PhD?.....	364
8.6.1 Prevalence, associations and natural history of pain in multiple sclerosis 364	
8.6.2 Neuroimaging correlates of pain syndromes in multiple sclerosis	364
Published evidence	364
Approach to MRI imaging experiments.....	365
Approach to resting state fMRI analysis	366
8.7 Potential improvements in methodology.....	367
Longitudinal study design	367
Neuropsychological testing	367
Quantitative Sensory Testing (QST)	368
Structural imaging	368
Resting state functional imaging	369
8.8 Selected possible future research questions	370
Epidemiology	370
Clinical correlates of the presence of pain in RRMS	370
Are the current experimental findings reproducible in RRMS, as well as other MS phenotypes and other related disorders (eg NMO)?	371
Mechanistic significance of study findings	371
Participant satisfaction with study involvement.....	372
Appendix: Scopus Search Terms	377
Appendix: Study newsletters.....	393
Overview	393

Appendix: Exploration of BOLD response to extrinsic thermal noxious stimulation: task fMRI	400
8.9 Introduction	400
8.10 Methods	400
8.10.1 Subject assessment	400
8.10.2 Thermal stimulation	400
Set-up and installation of Pathway device	400
Note: use of wave guide to allow connection to control room	401
Thermode	402
Interface with study participant.....	402
Psychophysical session prior to imaging	402
8.10.3 Scan session	404
Imaging acquisition.....	404
fMRI task	404
Note: use of goggles during scan session.....	404
Note: use of physiological noise modelling during session	405
Analysis.....	406
Clinical variables.....	406
Task fMRI	406
8.11 Results	408
8.11.1 Recruitment and Patient data	408
8.11.2 Psychophysical assessment	408
Individual patient issues	409
8.11.3 Mean BOLD signal in pain group and control group	409
8.11.4 Statistical Contrasts between control and pain groups.....	411
8.12 Discussion	412
8.12.1 Weaknesses	412
Variability in pain scores	412
Stimulus-correlated motion.....	412
Average pain ratings across ten trials.....	412
8.12.2 Strengths.....	412
Novelty.....	412

Nature of stimulus	413
8.12.3 Perspectives in relation to resting state analysis	413
8.12.4 Conclusions	414
References	415

Table of Tables

Table 1: Use and perceived effectiveness of selected pharmacological therapies for pain:.....	6
Table 2: Cortical localisation of functions tested in neuropsychological assessments	22
Table 3: Planned use of systematic review data in design of clinical study	51
Table 4: Database Search Strategies	64
Table 5: Characteristics of Included Studies.....	72
Table 6: Meta-regression analysis of study- and population- level associations with pain prevalence estimates.....	82
Table 7: PubMed search strategy	93
Table 8: Summary of experimental studies identified by this review.....	101
Table 9: Quality assessments of experimental studies.....	105
Table 10: Description of MRI imaging techniques in included studies.....	108
Table 11: Pain syndromes assessed in included studies	111
Table 12: Location of demyelinating lesions linked to pain syndromes in identified studies.....	114
Table 13: Classification of medication consumption.....	125
Table 14: Overview of neuropsychology instruments, and duration of administration	135
Table 15: Overview of putative functions tested by individual neuropsychology tests	141
Table 16: Targetted Quantitative Sensory Testing.....	143

Table 17: Primary reasons for exclusion of potential participants with MS (decreasing frequency)	148
Table 18: Participant Medication	152
Table 19: Demographics and Medication in Participants with and without neuropathic pain	159
Table 20: Psychological Comorbidity in participants with and without neuropathic pain	160
Table 21: subjects meeting cut-offs for major depression, and generalized anxiety	161
Table 22: Memory and Processing Speed in participants with and without neuropathic pain	162
Table 23: Executive Functions in participants with and without neuropathic pain .	164
Table 24: Demographics and testing site in quantitative sensory testing groups.....	165
Table 25: Quantitative sensory testing in healthy volunteers, and people with MS	167
Table 26: Quantitative Sensory Testing in people with MS, with and without neuropathic pain	168
Table 27: Quality of life: Comparison of subjects with and without pain	172
Table 28: Exploration of demographics and matching: subjects not receiving adjuvant analgesics.....	173
Table 29: Exploration of psychological and affective morbidity scores: subjects not receiving adjuvant analgesia	174
Table 30: Exploration of BICAMS measures: subjects not receiving adjuvant analgesia	175
Table 31: Exploration of executive functions: subjects not receiving adjuvant analgesia	177

Table 32: Exploration of quantitative sensory testing: subjects not receiving adjuvant analgesics	178
Table 33: Demographics and Medication in participants with varying severities of neuropathic pain	179
Table 34: Exploration of correlations between pain severity measures (Spearman's Rho, n=31)	181
Table 35: Psychological and psychiatric comorbidity: correlation with pain severity	182
Table 36: Memory and Processing Speed: correlation with pain severity.....	182
Table 37: Executive function measures: correlation with pain severity	183
Table 38: Quality of Life: Correlation between pain severity and quality of life measures.....	184
Table 39: Correlations of pain severity with pain interference scores.....	185
Table 40: Specifications of structural imaging sequences	189
Table 41: Comparison of tissue class volumes in pain and control groups	216
Table 42: overall lesion volume, whole brain and brainstem – comparison between pain group and control group.	217
Table 43: anatomical location of T2 hyperintense lesions more likely in pain group than controls	222
Table 44: Localisation of VBM result; exploratory threshold $p < 0.001$ uncorrected	224
Table 45: Correlations of tissue class volumes with pain severity	228
Table 46: Spearman correlations between automated cord cross-sectional area estimates, and intracranial volume (n=45)	237

Table 47: Correlations between cord cross-sectional area estimates, intracranial volume, disability score and disease duration (n=45)	239
Table 48: variations in cervical cord cross-sectional area measures by gender (n=45)	240
Table 49: Comparison of CC-CSA in study participants with and without neuropathic limb pain.....	241
Table 50: Structures manifesting higher functional connectivity to ACC seed in correlation with increasing T2 lesion volume	269
Table 51: Comparison of demographics, survey responders and non-responders...	277
Table 1: Psychophysical data	408

Table of Figures

Figure 1: Inter-relationship of pain with emotional/affective factors, and cognitive factors.....	10
Figure 2: Key components of descending pain modulatory system (DPMS).....	11
Figure 3: Flowchart describing selection of studies.....	70
Figure 4: Overall prevalence of pain in multiple sclerosis (seventeen studies).....	75
Figure 5: Overall prevalence of headache, stratified by timeframe of assessment (seventeen estimates)	76
Figure 6: Prevalence of specific pain syndromes.....	78
Figure 7: Reported aetiology of pain syndromes	79
Figure 8: Prevalence of pain occurring at multiple body sites.....	80
Figure 9: Flowchart describing selection of studies.....	98
Figure 10: Probability distribution of neuropathic pain (n=31) (JPEG data shown due to memory constraints).....	169
Figure 11: Probability distribution of non-painful neuropathic sensory symptoms (n=16)(JPEG data shown due to memory constraints)	170
Figure 12: Mask of supraspinal descending pain modulatory system used in this study	194
Figure 13: process of manual ICV estimation.....	195
Figure 14: Illustration of semi-automated MS lesion segmentation process	200
Figure 15: manual and automated estimates of cervical spinal cord cross-sectional area (spinomedullary junction level).....	209
Figure 16: correlation of automated and semi-automated ICV estimates.....	212

Figure 17: Bland-Altman plot comparing manual and automated ICV estimates (n=28).....	213
Figure 18: Scatterplot of first and second white matter hyperintensity manual lesion mask (n=9).....	214
Figure 19: Bland-Altman plot comparing two manually edited lesion masks by single rater (n=9).....	215
Figure 20: Lesion distribution probability maps: Whole group, pain group and control group (n=45 total)	219
Figure 21: Difference in lesion probability maps, pain and control group, by groupwise subtraction	221
Figure 22: Brainstem lesion topography - locations of T2 hyperintense lesions more likely in pain group than controls.....	223
Figure 23:Voxel based morphometry - grey matter volume greater in control group than pain group; exploratory threshold $p<0.001$ uncorrected	225
Figure 24: Voxel based morphometry: grey matter volume greater in pain group than control group; exploratory threshold $p<0.001$ uncorrected.....	226
Figure 25: scatterplot of manual and automated CSA estimates at P2.5 (spinomedullary junction level) (n=15).....	233
Figure 26: Bland Altman plot comparing manual and automated CSA estimates at P2.5 (spinomedullary junction level) (n=15)	234
Figure 27: Scatterplot comparing manual and automated CSA estimates (C2/3 level)	235
Figure 28: Bland Altman plot comparing manual and automated techniques for CSA estimation (C2/3 level)	236

Figure 29: Example of sudden head motion during resting state fMRI acquisition	251
Figure 30: Examples of structured noise sources identified in resting state data using ICA.....	253
Figure 31: Seed mask and Region of Interest masks used in this chapter	257
Figure 32: Mean functional connectivity of rostral ACC seed, separately calculated in pain and control groups.....	263
Figure 33: Differential functional connectivity between ACC seed and PAG, in pain and control groups.....	264
Figure 34: Differential functional connectivity between ACC seed and PAG in pain and control groups, after correction for T2 hyperintense lesion volume	265
Figure 35: Differential functional connectivity between rostral ACC seed and bilateral DLPFC region of interest, between pain and control groups.....	266
Figure 36: Trend to positive correlation of rostral ACC: PAG connectivity, with pain severity at time of imaging.....	267
Figure 37: Trend to negative correlation of rostral ACC: DLPFC connectivity, with pain severity at time of imaging.....	268
Figure 38: Structures demonstrating positive correlation between T2 hyperintense lesion volume, and functional connectivity with rostral ACC seed.....	270
Figure 39: overall satisfaction with participation (0= "not at all", 7 = "completely")	277
Figure 40: satisfaction with information given (0 = "not at all", 7 = "completely")	278
Figure 41: satisfaction with assessments at Rowling Clinic (0 = "not at all", 7 = "completely")	279

Figure 42: satisfaction with aspects of MRI imaging at CRIC (0 = "not at all", 7 = "completely").....	281
Figure 43: perceived difficulty associated with functional MRI procedures and equipment. (0 = "not at all ", 7 = "complete ")	282
Figure 44: Would you be interested in being contacted about giving advice on how future studies might be designed? (n=29)	287
Figure 45: would you consider taking part in a similar study in the future? (n=30)	287
Figure 1: Pathway device set up in mock MRI room, Clinical Research Imaging Centre	401
Figure 2: Mean BOLD signal in control group, n=16. Z=2.3, p=0.05 (moderate pain severity stimulus: "Temp 2").....	410
Figure 3: Mean BOLD signal in pain group, n=29. Z=2.3, p=0.05 (moderate pain severity stimulus: "Temp 2").....	411

Abbreviations

ACC	Anterior Cingulate Cortex
ALS	Amyotrophic Lateral Sclerosis
BBR	Boundary Based Registration (packaged in FSL, see FSL)
BET	Brain Extraction Tool (packaged in FSL, see FSL)
BICAMS	Brief International Cognitive Assessment for Multiple Sclerosis
BOLD	Blood Oxygen Level Dependent
BPI	Brief Pain Inventory
BPV	Brain Parenchymal Volume
CSF	Cerebrospinal Fluid
DKEFS	Delis Kaplan Executive Function Score
DLPFC	Dorsolateral Prefrontal Cortex
DMN	Default Mode Network
DWI	Diffusion Weighted Imaging
ECAS	Edinburgh Cognitive ALS Screen
EDSS	Expanded Disability Status Scale
EFNS	European Federation of Neurological Sciences
EPI	Echo Planar Imaging (refers to MRI acquisition)
FEAT	FMRIB's Expert Analysis Tool (packaged in FSL, see FSL)
FLAIR	FLuid Attenuated Inversion Recovery (MRI sequence)
FLIRT	FMRIB's Linear Image Registration Tool
fMRI	Functional Magnetic Resonance Imaging

FMRIB	fMRI of the Brain (research group/laboratory, Oxford University)
FNIRT	FMRIB's Nonlinear Image Registration Tool
FSL	FMRIB Software Library Suite of image analysis software/statistical software; open source, University of Oxford (see FMRIB)
FSLVIEW	FSL image viewer/manipulation program (see FSL)
FSS	Fatigue Severity Scale
GLM	General Linear Model
GM	Grey Matter
GUI	Graphical User Interface
HADS	Hospital Anxiety and Depression Scale
IASP	International Association for the Study of Pain
ICA	Independent Components Analysis
ICV	Intracranial Volume
IQ	Intelligence Quotient
MELODIC	Multivariate Exploratory Linear Optimized Decomposition into Independent Components (packaged in FSL, see FSL)
MNI	Montreal Neurological Institute
MNI152	Montreal Neurological Institute standard space brain template (based on 152 brains)
MRI	Magnetic Resonance Imaging
MS	Multiple Sclerosis
NART	National Adult Reading Test

OFC	Orbitofrontal Cortex
PAG	Periaqueductal Grey matter
PASAT	Paced Auditory Serial Addition Test
PCA	Principal Components Analysis
PCC	Posterior Cingulate Cortex
PCS	Pain Catastrophizing Scale
PPMS	Primary Progressive Multiple Sclerosis
PSQI	Pittsburgh Sleep Quality Instrument
pwMS	People/person with MS
QOL	Quality of Life
QST	Quantitative Sensory Testing
R (program)	Statistical analysis program/language
R studio	User interface for R
rACC	Rostral Anterior Cingulate Cortex (see ACC)
ROI	Region of Interest
RRMS	Relapsing Remitting Multiple Sclerosis
RVM	Rostral Ventromedial Medulla
SF36	Short Form 36 (Quality of Life instrument)
sLANSS	Self-Report Leeds Assessment of Neuropathic Symptoms and Signs
SNRI	Serotonin-Norepinehrine Reuptake Inhibitor
SPM	Statistical Parametric Mapping
SPMS	Secondary Progressive Multiple Sclerosis
SSRI	Selective Serotonin Reuptake Inhibitor
SVC	Small volume comparison
T1W	T1 weighted MRI
T2W	T2 weighted MRI
TCA	Tricyclic Antidepressant

TFCE	Threshold Free Cluster Extent
VBM	Voxel Based Morphometry
VLPFC	Ventrolateral Prefrontal Cortex
WM	White Matter
WUR	Wind Up Ratio

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Declaration

I declare that this thesis has been composed solely by myself and that it has not been submitted, in whole or in part, in any previous application for a degree.

Except where stated otherwise by reference or acknowledgement, the work presented is entirely my own.

Peter Foley

Abstract

Background:

Pain is frequently reported by people with multiple sclerosis (MS). It has been associated with decreased quality of life, psychiatric morbidity, interference with day to day activities, and frequent healthcare attendance. It has been reported by people with multiple sclerosis to be one of their most important symptoms, and available treatments are limited in their effectiveness.

Despite this, our understanding of the epidemiology and mechanisms of pain in people with MS are limited.

Our understanding of the interactions of central nervous system mechanisms and pain states overall is growing. However, the application of this knowledge to MS is incomplete. Previous studies have shown that the descending pain modulatory system (DPMS) is an endogenous network of cortical and subcortical brain structures which act to limit, or accentuate, an individual's perception of pain, via descending brainstem pathways. Associated clinical measures include depression, anxiety, and cognitive flexibility. Our understanding of the function or dysfunction of this system in MS is limited. We do not know if the MS disease process may adversely affect the structure or function of the DPMS.

Hypothesis:

In people with neuropathic limb pain in relapsing remitting MS (RRMS), compared to people with RRMS who do not have pain, there will be disruption of the endogenous descending pain modulatory system. This will manifest as impaired descending inhibition of pain.

Aims and Methods

Establishing the background using systematic reviews:

The first aim of this thesis was to establish the prevalence, natural history and associations of pain (and pain syndromes) occurring in people with MS.

The second aim was to explore existing knowledge of how the MS disease process may contribute to pain states, using a systematic review of neuroimaging studies.

Prospective clinical study:

A case-control study of 47 people with RRMS was then carried out. 31 of these had neuropathic pain in the limbs, and 16 did not have pain. Using targeted assessments, function of the descending pain modulatory system was assessed in the following ways:

First: Detailed clinical, behavioural and neuropsychological assessment, focussing on cognitive, behavioural and affective features known to be closely related to the DPMS.

Second: MRI imaging of brain structure, focussing on the volume and location of MS lesions, as well as the volume of key grey-matter structures involved in the DPMS.

Third: Resting state functional MRI imaging of the brain, focussing on functional connectivity between the rostral anterior cingulate cortex and two other key DPMS structures (dorsolateral prefrontal cortex, and periaqueductal gray).

Results:

Systematic reviews:

Meta-analysis of existing prospective studies confirmed that pain is very common in MS, affecting about 63% of people with MS on average (95%CI between 55 and 70%). Many different types of pain contribute to this overall estimate. No significant associations with disease course or stage emerged.

Several neuroimaging studies have assessed people with MS-associated pain using MRI. These studies were often small, and with associated methodological issues. It is likely that location of MS lesions is implicated in aetiology of pain syndromes in some cases, though our overall knowledge is limited.

Prospective study:

In a prospective study, people with and without pain were matched for age and gender. Furthermore, groups were balanced for a range of other variables. The pain group more frequently received gabapentinoid medications.

The presence of pain was significantly associated with increased scores for depression, fatigue and catastrophising, as well as with specific impairments at neuropsychological assessment, including cognitive flexibility. Many of these impairments are directly relevant to existing models of the DPMS.

Overall volume of MS lesions was not different in people with pain, though lesions were more likely to occur in the brainstem. Some alterations of grey-matter volumes in people with pain which mirrored studies of pain disorders outside MS were found, but these did not involve structures key to the DPMS. Affected structures included trigeminothalamic nucleus (relative volume increase in pain group), posterior cingulate cortex and parahippocampal gyrus (volume decrease in pain group).

Functional connectivity of the rostral anterior cingulate cortex to the periaqueductal grey matter, a key structure in the descending modulation of pain, was stronger in the group without pain. Conversely, functional connectivity to the dorsolateral prefrontal cortex, repeatedly implicated in the DPMS and thought to be involved in cognitive evaluation and flexibility, was stronger in the pain group. MS lesion volume appeared to account for some of this difference in a multivariate analysis.

Limitations:

Key limitations of this work include cross-sectional design, small sample size, and number of statistical comparisons carried out.

Conclusions:

Systematic reviews examined the prevalence, natural history and associations of pain in MS, as well as examining existing neuroimaging studies which investigated how the MS disease process could contribute to pain states.

A prospective study found evidence of both emotional/affective and cognitive dysfunctions relevant to the hypothesis of dysfunction in the DPMS.

Higher likelihood of MS lesions in the brainstem could be relevant to DPMS function. Separately, there were structural grey-matter volume alterations reflecting those found in many pain studies outside MS. Importantly, however, these did not affect key DPMS structures.

Resting state functional MRI however demonstrated altered connectivity of core DPMS structures, which may be partly mediated by MS lesion volume. Functional connectivity findings could be consistent with the hypothesis of impaired descending pain inhibition, in people with relapsing remitting MS affected by neuropathic limb pain.

Lay Summary

Background:

Multiple Sclerosis (MS) is a common disorder which causes inflammation and cell death in the brain and spine. Pain is a common and important symptom for people with MS, however our knowledge of how common it is, and why it develops, is incomplete.

Brain and spine scans in people with MS often show abnormalities, including areas of damage referred to as lesions. Among other effects, MS lesions are thought to damage connections between different parts of the brain.

Previous studies (though not in people with MS) have identified pain-regulating mechanisms existing within the human brain. These have been described as the “descending pain modulatory system” – a network of brain structures which can interact to increase or decrease a person’s experience of pain. Problems with structure or function of this system can be seen on detailed MRI scans, but are also associated with a range of problems including anxiety, depression, and inflexible thinking styles.

No previous studies have assessed whether the descending pain modulatory system could be damaged by the MS disease process in people with MS-associated pain.

Hypothesis

In people with neuropathic limb pain associated with MS, compared to those without, there are impairments in the descending pain modulatory system which are linked to the MS disease process.

Aims:

Firstly, to establish what we currently know about how common pain problems are in adults with MS, who is affected by pain problems, and when they are affected.

Secondly, to establish what existing studies using scanning of the brain or spine have identified about why these pain problems happen.

Thirdly, to study people with a type of MS (relapsing remitting MS) who had nerve pain (neuropathic pain) affecting their limbs, and to compare them to people with the same type of MS who did not have neuropathic pain.

Specifically, I aimed to assess variables that are known to be relevant to individuals' own pain regulating mechanisms, and to study whether these varied between people with, and without, neuropathic limb pain. I also aimed to examine the structural effects of MS on the brain, and the functional connectivity between key parts of the DPMS.

Methods:

Firstly, a detailed review (“systematic review”) of existing studies was used to identify and assess articles which describe how common pain in MS is, and who it affects.

Secondly, a further systematic review was used to identify and assess articles which describe brain and spine scanning of people with MS who have pain problems.

Thirdly, a study comparing 31 adults with neuropathic limb pain in MS, and 16 adults with MS who do not have pain was carried out. Medical examination, questionnaires, psychology testing, and “MRI” (magnetic resonance imaging) brain scanning - looking at both brain structure and function – were used.

Results:

How common is pain in MS? Systematic review confirmed that it is very common, affecting over half of people with MS (about 63%, though there is a lot of variation between studies). There are multiple different types of pain, and they do not affect just one particular type of person with MS.

Do existing scan studies tell us the cause of pain in MS? Several studies have looked at scans in people with MS, but they have been relatively limited and have not given

us clear answers about why pain happens, in most cases. There is limited evidence that MS inflammation areas (“lesions”) in particular parts of the brain or spine may be linked to pain, in some cases.

Are there differences in factors related to pain regulation in a group of people with MS who have nerve pain, compared to a group who do not?

The group with pain showed some characteristics which could be associated with impaired ability to regulate pain. These included higher scores for depression, fatigue and negative thinking styles as well as limited mental flexibility.

MRI brain scans showed that the volume of MS lesions is not higher overall in the brains of people with pain. Volume of lesions in the brainstem was however higher in those with pain.

The volume of key structures involved in pain regulation was not different in those with, and without, pain.

When functional MRI was used to look at functional connections between these areas, however, connectivity between these areas was found to be altered in those with pain, compared to those without. In particular, those with pain had reduced connectivity between two key structures involved in pain regulation (the anterior cingulate cortex, and the periaqueductal gray matter in the brainstem). Volume of MS lesions appeared to account for at least some of this connectivity difference.

Discussion: This work confirms that pain in MS is common, and that existing scanning studies do not fully explain why it happens.

In a new study of people with relapsing remitting MS with and without neuropathic limb pain, those with pain were more likely to show depression, fatigue, or inflexible thinking styles. While brain structure (for instance volume of key brain structures, or volume of MS lesions) did not vary between those with and without pain, functional connectivity between key areas involved in pain regulation was altered. In this small study, these results could support the possibility of altered function in the descending

pain modulatory system in adults with neuropathic limb pain associated with relapsing-remitting MS.

Chapter 1 Introduction

Some relevant background to the thesis as a whole is discussed, before considering specific aims and hypotheses.

1.1 Background

1.1.1 Key definitions: pain

Definition of pain

Pain is a highly subjective experience. There is a complex relationship between a stimulus which may cause pain (nociceptive input) and the perception of pain (1).

For the purposes of this thesis, pain is defined as

“An unpleasant emotional and sensory experience, associated with tissue damage, or defined in terms of such damage”.

The definition is based on the International Association for the Study of Pain taxonomy taskforce (1994) (2). Pain is considered as a multifactorial experience with a range of components including physiological, affective, cognitive, behavioural and social (3).

Definition of neuropathic pain in this thesis

Throughout this thesis, neuropathic pain is defined as “pain caused by a lesion or disease of the somatosensory nervous system” (2). This definition is comparable to other proposed definitions, for instance “pain arising as a direct consequence of a lesion or disease affecting the somatosensory system”(4).

Central neuropathic pain is similarly defined in this thesis as “pain caused by a lesion or disease of the central somatosensory nervous system” (where central nervous system is defined as the brain and spinal cord) (2).

Definition of neuropathic limb pain in this thesis

Throughout this thesis, neuropathic limb pain is defined as neuropathic pain affecting one or more limbs. Other commonly-used terms including “dysaesthetic pain”, “neuropathic extremity pain”, and “central pain”, can allow for ambiguity in

interpretation (5). This term is favoured for clarity in terms of stated aetiology and location of pain.

1.1.2 Why is pain in multiple sclerosis an important research target?

A number of strands of evidence suggest that neuropathic pain in multiple sclerosis (MS) is an important research target. These include its prevalence, the impact on people who experience it, the importance to wider society in terms of healthcare utilization, and limited response to medication.

1.1.2.1.1 *Prevalence*

The prevalence of pain in multiple sclerosis, and of particular subtypes of pain, is discussed in more detail in Chapter Two. Overall pain prevalence is unclear, with estimates ranging widely from 29% to 86% (6, 7). Studies examining relationships of pain prevalence to clinical variables use differing patient samples and study design, and report inconsistent conclusions.

1.1.2.1.2 *Patient ratings of the importance of pain in MS*

People with multiple sclerosis have described pain as an important symptom in a number of studies.

In one study of 166 UK outpatients with multiple sclerosis, patients were asked to provide an estimate of which “functions” were most important to them (8). Thirteen “functions” were presented to patients, who were asked to rate them from 1 to 13 according to subjective value and relevance. Lack of pain was rated as the fourth most important priority to this group (regardless of disease duration). Walking, visual function, and speech were the only functions rated as more important. Functions rated as less important than pain included power and coordination of hands, thinking and memory, continence and mood (8).

In a further study of 612 UK adults with MS who experienced pain, a survey was used to examine experience and correlates of pain (9). On a numerical rating scale of 1-10, subjects were asked to rate “level of interference” attributed to specific MS symptoms. Pain was rated as causing the fourth most interference, after fatigue, sexual dysfunction and balance. It was reported to be associated with more

interference than several other symptoms including bowel or bladder problems, thinking, vision problems, tremor, and speech problems.

A further cross-sectional study of 117 people with multiple sclerosis admitted to a neurology inpatient unit in Denmark (10) identified that 32% listed pain as one of the most severe symptoms of MS.

Together, these data suggest that pain is a major problem for people with MS, that it interferes with daily life, and that it can be one of the most severe symptoms of MS.

1.1.2.1.3 Associations of decreased quality of life with pain in MS

Several authors have described links between presence or severity of pain in MS, and poorer quality of life.

Kalia and colleagues, writing in 2005, studied 99 Canadian outpatients with multiple sclerosis (11) using the SF-36 instrument to measure quality of life. The SF-36 generates subscores reflecting physical and mental aspects of quality of life (12). Chronic pain in this study was defined as a constant or near-constant pain in the preceding month, not related to other causes. The presence of chronic pain was significantly associated with decreased vitality and mental health scores, (5% and 1% significance levels respectively). Severity of pain was negatively correlated with role limitation by emotional processes ($p<0.05$), vitality, role limitation by physical processes and general health (all $p<0.01$); mental health and social functioning ($p<0.0001$).

Hirsh and colleagues, in a large 2009 study of 2994 US veterans with multiple sclerosis (13) used a postal survey to assess physical and mental health of participants, as well as presence and severity of pain. An adapted, shorter, version of the SF-36, termed the V-36 (12, 14) was used. In particular, they aimed to assess the association of both presence and impact of pain, with several variables. The prevalence of pain in the preceding four weeks was high (92% of participants). Eighty-five percent of the participants described that pain cause interference in daily activities, over the same time period.

Physical health measures were significantly associated with pain intensity, and perceived interference of pain with ability to complete daily activities (Pearson correlation -0.23 and -0.25 respectively, $p < 0.001$). Mental health measures were also significantly associated with both pain intensity and interference (Pearson correlation -0.38 and -0.42 respectively, both $p < 0.001$), to a greater degree than physical health. The strongest predictors of pain interference (i.e. the phenomenon of pain interfering in day-to-day activities) were pain intensity ($\beta = 0.73$), physical health ($\beta = -0.07$) and mental health ($\beta = -0.03$). There was a statistical interaction between pain intensity and physical health, underlining the complexity of these phenomena.

Presence of pain was also associated with lower scores (indicating worse quality of life) in all subscales of the SF-36 in a study of 50 people with MS and pain, 50 people with MS without pain, and 50 healthy volunteers (15).

Together, these data suggest that quality of life measures, including measures of both physical and mental health, are repeatedly negatively associated with the presence and severity of pain in multiple sclerosis.

1.1.2.1.4 *Healthcare utilization for pain in MS*

Several studies demonstrate that people with MS (pwMS) who experience pain, frequently access healthcare because of pain.

In one study of 125 community-dwelling people with MS in the north-western USA who reported recent or current pain problems (16), participants were asked about health care visits specifically for pain during the past six months. 75% of participants reported accessing health care providers for pain in the preceding six months, with a mean number of total visits 9.7 in six months (SD 14.4, range 0-81). The three specialties most commonly accessed were primary care (62% of participants, mean number of visits 2.1), speciality physician (47%, number of visits 1.8) and physical or occupational therapy (33%, number of visits 3.5).

In a study of 61 community-dwelling adults with MS and pain in Australia, around half reported accessing their GP because of pain within the preceding 12 months (32/61, 52.5%). Other specialists most frequently consulted over the same time

period included neurologists (29/61, 47.5%) and physiotherapists (21/61, 34.5%) (17). At follow-up after 7 years, frequency of access to the same practitioners had increased in all cases (after seven years, percentages accessing health care for pain were: GP 54.1%, physiotherapist 48.6% and neurologist 62.2%).

These American and Australian studies both describe substantial healthcare utilization related to pain, in people with pain related to multiple sclerosis. Healthcare utilization was observed across a range of specialties and may increase over time. These data could be consistent with MS-associated pain being experienced as distressing (leading patients to need to access healthcare), or that it is not easily resolved (leading to repeated visits). Economic costs have not been estimated, but may be considerable.

1.1.2.1.5 *Treatment of pain in MS*

1.1.2.1.5.1 Perceived efficacy of pharmacological therapy

Medication has been reported in several studies to be of limited efficacy in treating pain, for people with MS. This may, in part, relate to limited mechanistic understanding.

A 2001 study of 83 members of the USA national MS society (18), of whom 90% reported experiencing pain, asked participants to rate the eight techniques which were most effective, and least effective, in managing their pain. The investigators used a survey (by post or in person). Medication overall was rated as effective by 45% of participants. More specifically, anti-inflammatories were rated as effective by 13%, opioids by 5%, antidepressants by 4%, and anticonvulsants by 3%. Interestingly, 48% of participants listed medication as among the least effective techniques for managing their pain.

One study of community-dwelling adults with MS in the northwestern USA (n=125 with current or recent pain (16) specifically examined participants' perceptions of the efficacy of specific pharmacological and non-pharmacological therapies. Participants were asked to rate the effectiveness of various pharmacological and non-pharmacological therapies in relief of their own pain, on a scale 0-10, from "no pain

relief” to “complete pain relief”. Data regarding patients’ use of, and perceptions of, selected therapies relevant to this study is reproduced below (Table 1).

Table 1: Use and perceived effectiveness of selected pharmacological therapies for pain:

reproduced from Ehde et al, 2015

Medication	% of participants who had ever used	% of participants still using	Pain relief rating (0 “none” to 10 “complete”, see text) (mean, SD)
Paracetamol ¹	78.4%	58.2%	4.8 (2.6)
NSAID	82.4%	69.9%	5.1 (2.7)
TCA	27.2%	38.2%	3.9 (2.9)
Gabapentin	23.2%	62.1%	5.1 (2.8)
Carbamazepine	17.6%	40.9%	4.2 (4.2)
Opioids	42.4%	37.7%	6.6 (2.9)
¹ Paracetamol described as Acetaminophen in this study in the USA NSAID = Non Steroidal Anti Inflammatory Drug TCA = Tricyclic Antidepressant SD = Standard Deviation			

1.1.2.1.5.2 Polypharmacy

Polypharmacy is also common among patients with multiple sclerosis. This may lead to an increased risk of side effects, and of medication interactions.

In a recent retrospective study of 1090 patients with MS attending a specialist inpatient rehabilitation unit in Norway (2009-2012), 342 of 1090 (31%) used at least one adjuvant analgesic (19). Of the 1090 subjects studied, mean number of medications administered was 5.4, with concomitant use of adjuvant analgesic agents along with at least one other drug acting on the central nervous system also being common (19). These data are comparable to other studies, for instance Kalia and colleagues’ study of 102 people with MS attending an outpatient MS clinic in Toronto between 2000 and 2002 (11). Of these, 26% had received treatment for pain in the last month. Fifty percent of those receiving pain therapy were receiving more than one type of treatment (though these estimates included non-pharmacological therapies).

Together, these data suggest that use of medications for pain in MS is common, and that perception of utility is variable, though does not suggest marked benefit. Polypharmacy is common and could in itself contribute to adverse outcomes.

1.1.3 Epidemiology of pain in multiple sclerosis

There is limited understanding of how often pwMS are affected by pain, which MS patient groups suffer most frequently from pain, or of the influence of study methodology on pain estimates. For example, some studies have found that the presence of pain in MS is associated with increasing disability and disease duration, (7, 20-23) whereas others have found no such relationship. (24-27) The reasons for such differences are poorly understood. Lastly, the natural history of pain during the disease course is uncertain.

One previous systematic review carried out in 2007 (5) usefully explored some of these issues. The authors did not however examine the literature published in languages other than English, and did not use weighted meta-analysis to calculate prevalence estimates. Therefore confidence intervals for estimates are not available, and estimate heterogeneity has not been quantified, nor formally explored.

Better understanding of the prevalence, and natural history, of MS-related pain could assist clinicians and healthcare planners in estimating the likely extent of this problem, and the size of the population affected. Improved understanding of patient groups affected might also assist clinicians in focussing assessment on patient groups who are particularly at risk of pain.

Furthermore, better understanding of the epidemiology of pain in MS could improve understanding of symptom mechanisms, and potentially contribute to development of targeted treatment strategies. For instance, CNS inflammation and neurodegeneration may be most likely to occur in early and late disease, respectively. (28) Variation in pain prevalence across the disease course could suggest links to neuroinflammatory or neurodegenerative processes.

1.1.4 The descending pain modulatory system (DPMS)

Overview of DPMS

As discussed, the experience of pain is not straightforward, and is not linearly related to nociceptive input (29). The nociceptive input, environment, and behavioural state of the person experiencing pain are all particularly important, and interact to modulate the experience of pain (30).

1.1.4.1.1 *Descending pain modulation systems: an example in acute injury*

The experience of acute pain related to an injury may differ significantly according to a range of variables. The relatively straightforward example of a footballer incurring a leg injury during a tackle can be used to highlight some relevant clinical, behavioural and psychological themes.

Firstly, the nature of the injury may vary, leading to varying *nociceptive input* (for instance minor physical impact in comparison to significant trauma). The *environment* in which the injury is incurred (for instance during a crucial moment in a major competitive football match) may also critically modulate the severity of perceived pain. The experience of the pain associated with the injury may be further modulated by *cognitive* factors – such as the footballer’s appraisal of the significance of the injury. *Emotional and affective* variables (such as the player’s mood) may also importantly modulate the experience of pain (31).

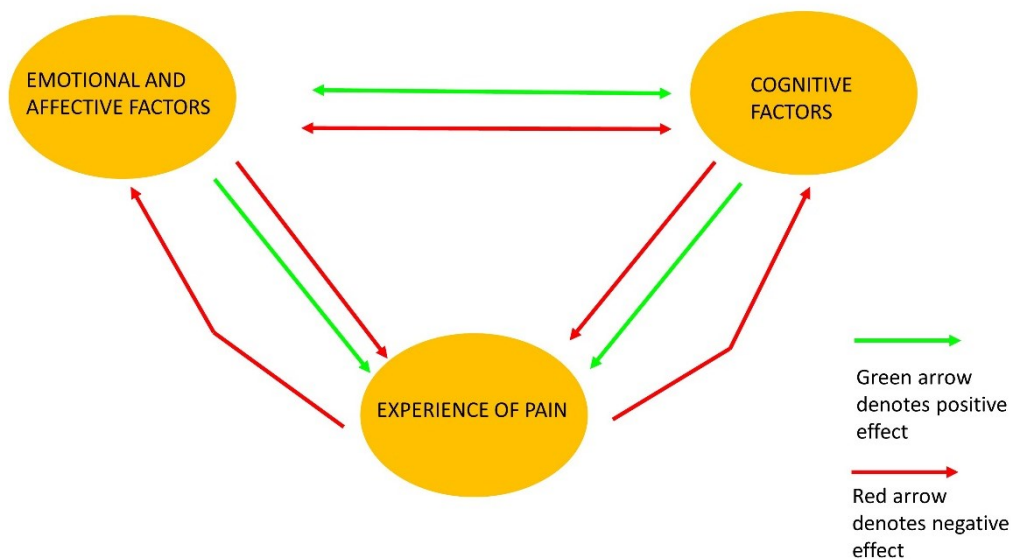
All of the above factors may reduce, or increase, the severity of perceived pain. Pain-related behaviour may then be modulated.

Emotional/affective factors and cognitive factors, can exert bidirectional modulatory influences on the experience of pain.

1.1.4.1.2 Descending Pain Modulatory System: role of cognitive and emotional/affective factors

This complex inter-relationship between emotional/affective factors, cognitive factors and the experience of pain is summarised below (Figure 1) (adapted from Bushnell et al (31)).

Figure 1: Inter-relationship of pain with emotional/affective factors, and cognitive factors
(adapted from Bushnell et al)

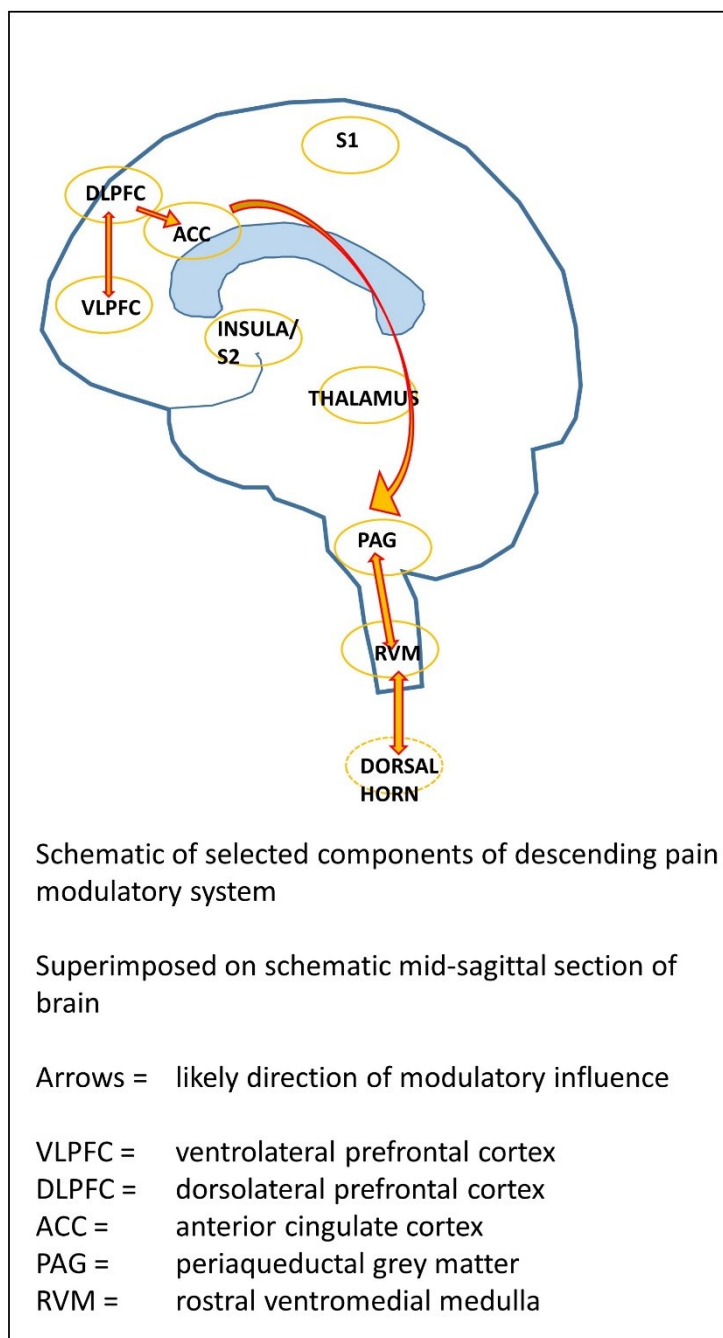


All of these factors are relevant to the descending pain modulation system (DPMS).

In summary, forebrain and limbic circuits are recognised to exert modulatory input to the descending pain modulation system (30). Particularly important areas include Prefrontal Cortices, Amygdala, anterior insula, and Anterior Cingulate Cortex (30). These exert top-down influences via brainstem circuits which in turn are thought to exert influences on the dorsal horn of the spinal cord. Key brainstem circuits are centred on the Periaqueductal Gray (PAG) and Rostral ventromedial Medulla (RVM) (1, 30, 32). Endogenous opioids as well as serotonergic signalling are thought to be key to this system (30).

The key neuroanatomical structures involved in the DPMS are highlighted below (Figure 2) (after Tracey et al, and Wiech et al (1, 33)).

Figure 2: Key components of descending pain modulatory system (DPMS)



1.1.4.1.3 Descending pain modulation in chronic pain

In chronic pain, as found in MS, emotional/affective and cognitive factors are crucially important (30).

The following section aims to summarise studies relating findings relevant to DPMS function or dysfunction in people with MS. There is a particular focus on studies describing anxiety, depression, cognitive dysfunction and sensory processing, with particular emphasis on studies detailing these variables in relation to the presence or severity of pain in MS, where available.

Is there evidence consistent with DPMS dysfunction in pain related to MS?

1.1.4.1.4 *Depression in MS*

The prevalence of major depression in MS has repeatedly been found to be high, with several studies reporting a higher prevalence of depression in MS than in healthy controls, or in subjects with other chronic disease (34).

A recent large Canadian study (35) compared the incidence and prevalence of psychiatric disorders in the MS population, and in controls who were matched for geographic area, age and sex, using population-based administrative health data. Case definitions of psychiatric disorders used coding based on ICD-9 or ICD-10 criteria from 1995 to 2005. Case definitions of multiple sclerosis used coding based on the same criteria. 44,452 MS cases, and 220,849 matched controls were identified. In 2005, the annual incidence of depression was 979 per 100,000 people with MS (0.98%), compared to 0.72% in the matched population without MS. Age-standardized incidence of depression was 71% higher in the MS population than the matched population. Age-standardized prevalence of depression was also higher in the MS population than the matched population (20.1% vs 11.9% respectively).

Three prospective studies using a standardised psychiatric interview have similarly estimated the prevalence of major depression to be between 42 and 54% (sample size between 50 and 221 people with MS) (34). A fourth larger study of 739 people with MS completing a mailed survey (36) used the Centre for Epidemiological Studies' Depression Scale (CES-D), and found that 42% of the sample had "clinically significant depressive symptoms" (CES-D score >16.0) and 29% scored in a range suggesting moderate or severe depression (CES-D score >21). In this sample, younger age, less educational attainment and lack of social support were associated with depressive symptoms.

1.1.4.1.5 *Anxiety in MS*

Incidence and prevalence of anxiety in the Canadian population-based study described above (37) was higher in the MS population than the matched population (crude annual incidence 0.64% vs 0.42%; crude prevalence 8.7% vs 5.1%).

A prospective interview study of 140 clinic attenders with MS in Toronto (38) administered the structured clinical interview for DSM-IV disorders (SCID-IV) (39) as well as the Hospital Anxiety and Depression Scale (HADS) (40). Lifetime prevalence of anxiety disorders using the SCID-IV was 35.7%, and point prevalence 14%. Anxiety disorders were reported to include panic disorder, social phobia, specific phobia, obsessive compulsive disorder, and generalized anxiety disorder. Using a threshold score of 10 on the HADS, 20.7% of the sample were found to have elevated anxiety scores, and 10.7% elevated depression scores.

1.1.4.1.6 *Inter-relationship of anxiety and depression in MS*

In a comparison of subjects with (n=50) and without (n=90) anxiety disorders, a diagnosis of major depression was more likely in those with anxiety (62.0% vs 22.2%, $p=0.0001$), whereas cognitive impairment was more common in those without anxiety (16.3% in those with anxiety, 28.9% in those without, not attaining statistical significance $p=0.1$). Mean HADS anxiety score in those diagnosed with anxiety using the SCID-IV interview was 8.8 (SD 4.6), and in those without anxiety 4.5 (SD 2.8).

Taken together, these data suggest that significant depressive symptoms affect at least 20% of people with MS (35), with some estimates being considerably higher (34, 36). Point prevalence of any anxiety disorder in MS has been reported to be between 8 (35) and 14% (38), with the two commonly coexisting (38).

1.1.4.1.7 *Anxiety and depression in MS-related pain disorders*

While not all studies examining the experience of people with MS have evaluated the presence or otherwise of affective symptoms (23), symptoms of anxiety and depression have repeatedly been found to be associated with presence and severity of pain.

Drulovic and colleagues administered a structured questionnaire to 650 outpatients with MS at several MS centres in Serbia, and Bosnia-Herzegovina (41). The severity of depressive and anxiety symptoms were rated using the Hamilton Rating Scale for Depression (HDRS) (42) and the Hamilton Rating Scale for Anxiety (HARS) (43). Mean scores for both depressive and anxiety symptomatology were higher in the pain group (patients with pain n=432, patients without pain n=218). For depressive symptomatology, mean HDRS score in those without pain was 9.3, and those with pain 13.8, (a threshold score >9 was suggested as consistent with mild depression). For anxiety symptomatology, mean score in those without pain was 8.5, and those with pain 13.0, (a threshold HARS score of >17 was suggested as consistent with mild anxiety).

Kalia and colleagues (11) dichotomized their group of 99 clinic attenders with MS around median HADS (40) anxiety score (median 7) and depression score (median 5) and found that median pain scores (assessed using the SF36 instrument (12)) were significantly higher in groups with higher anxiety scores, and those with higher depression scores.

Archibald and colleagues (44) applied the Mental Health Inventory (MHI) (45) in 85 patients with MS who underwent structured interviews in an MS referrals unit with a focus on pain. Global MHI scores were used, with higher scores reflecting better mental health status (highest possible score 226). The MHI scores were significantly lower in the group with pain, than the group without (mean 159.2 vs mean 170.4, $p<0.05$). MHI scores were below the mean score of the normative sample for this instrument (45), and the mean MHI score of the pain group fell within the lowest quartile of distribution of normative scores.

Pain and depression are frequently comorbid in MS. Alschuler and colleagues (46) used a postal questionnaire study of 161 community-dwelling adults with MS in the USA. Pain and depression were found to frequently coexist, with, in particular, pain being frequently found in people meeting diagnostic criteria for depression (using the PHQ-9 instrument). They describe that, of persons experiencing any pain, 11-34% met depression criteria. A slightly higher number (15-37%) met depression criteria when only pain of at least moderate severity was considered (46). Participants with pain were between two and four times more likely to meet depression criteria than those without.

The above data are consistent with increased depressive and anxiety symptomatology in people with pain associated with MS, in comparison to those who do not have pain. Most studies examined people with pain of different aetiologies.

1.1.4.1.7.1 Anxiety and depression in pain disorders other than those associated with MS

Data from pain disorders outside the context of MS are also consistent with the above findings. Painful physical symptoms have been reported to occur in between a half and two thirds of patients with major depressive disorder, and major depressive disorder has been reported in up to half of people receiving specialist treatment for chronic pain (47). It is not certain whether the experience of depression may predispose to pain, whether the experience of pain may predispose to depression, or whether there is a separate independent modulator (47).

Anxiety is likewise highly prevalent in people with chronic pain. In people with chronic pain being treated at tertiary centres, prevalence of any anxiety problem has been estimated at between 7 and 28%, while generalised anxiety disorder has been found to be present in up to 20% (47).

Longitudinal population-based studies have suggested that pre-existing mood disorders increase risk for developing chronic pain (people with depression but without pain are approximately twice as likely to develop chronic musculoskeletal pain as people without depression or pain). Similarly some evidence suggests that anxiety disorders may precede the onset of pain. In cross-sectional studies or clinical

practice, however, it is not always possible to determine direction of causality (if any) (29). Depression and pain may influence each other by a range of mechanisms (on the biopsychosocial “spectrum”) ranging from potential shared biochemical pathways, to effects on cognition, behaviour and the individual’s environment (46).

1.1.4.1.8 *Cognition in MS: background*

The prevalence of cognitive problems in MS is appreciable, though deficits may not always be readily clinically apparent. Estimates depend on the instruments used and the population tested. In a population cohort, cognitive impairment on neuropsychological assessment may be present in 35% of people with MS, whereas in a clinic cohort, up to 60% (48). Extensive research has focussed on the assessment (and potential for treatment) of cognitive dysfunction in MS. The ecological validity of tests (in other words the impact of tested functions on a person's capabilities in day to day life, or on quality of life) is not always known (48).

A core deficit in MS is information processing speed, with anterograde episodic memory, and executive function the next most frequently affected (49). Cognitive impairments can affect people with any stage of MS, including early MS and Relapsing Remitting disease (50), though they are thought to be more common in people with progressive disease of longer duration (51). No proven pharmacological or non-pharmacological treatment exists for cognitive impairment in MS (48)

In a recent large study of 1500 Israeli patients with MS using a computerized cognitive battery (51), the authors tested multiple cognitive domains including verbal and non-verbal memory, executive functions, visual spatial perception, verbal fluency, attention, processing speed and motor skills. Patients with significant depressive or anxiety symptomatology were excluded (noting the appreciable prevalence of affective disorders in MS discussed above). The authors used a range of tools generating 65 outcome parameters. Some tools from previous batteries were used, while many were novel.

This cross-sectional study recruited patients with variable disease durations, and reported that while cognitive impairment was more pronounced in people with SPMS and longer disease durations, the pattern of cognitive impairment was similar in all groups of patients. The most frequently impaired functions were information processing speed and executive function. Although people with significant anxiety or depression on the basis of Hamilton questionnaires were excluded, sub-threshold anxiety or depression scores were not accounted for. In addition pain, fatigue and sleep were not discussed (51).

1.1.4.1.9 *Inter-relationship of cognition, pain and other symptoms in MS*

The role of cognitive factors in pain related to MS is incompletely understood. There is likely to be a complex inter-relationship between cognitive, affective/emotional variables, and pain (Figure 1). This inter-relationship has not however been extensively studied. Reliable separation of cognitive and emotional factors is extremely difficult. For example a key aspect of depression is cognitive distortion (negative appraisal of the self, the world and the future) (52)). As has been discussed above, both affective and cognitive problems are prevalent in people with MS.

In a small study (53) of 48 patients with MS and healthy controls (university students), participants underwent a cognitive battery along with other tests aiming to establish symptoms of depression and fatigue. Cognitive assessment focussed on information processing speed and measures of memory, including the California Verbal Learning Test (54) and digit span (including reverse digit span) (55). Patients with known unipolar or bipolar depression were excluded. The authors specifically focussed on processing speed, felt to be a key deficit in MS. Slower information processing was associated with higher depressive symptomatology, impaired verbal learning, higher fatigue scores and poorer digit span.

Evidence of cognitive dysfunction in other chronic pain disorders includes one study of 30 people with fibromyalgia compared to 30 healthy controls (56). In particular, the authors found deficits in a range of cognitive variables measuring attention, and working memory. The fibromyalgia group reported much worse quality of life, depression and sleep. After accounting for differences in pain, differences in cognition were no longer apparent, leading the researchers to suggest that these differences may be accounted for by presence of pain. Effect of medication was examined by a contrast between people with fibromyalgia who were taking opioids and those who were not (n=9 and 21 respectively). The authors found that those taking opioids manifested better cognitive functions, though a small sample size was noted. Potential effects of other drugs including tricyclics (46.7% of fibromyalgia group vs 6.7% of control group) were not explored.

1.1.4.1.9.1 Pain catastrophising and appraisal of pain

Pain catastrophising is commonly related to the presence and severity of pain disorders (33) though has been infrequently assessed in studies of people with MS. Pain catastrophising has been defined as an exaggerated negative orientation towards painful stimuli during actual or anticipated pain experience (57), or (similarly), an exaggerated negative “mental set” brought to bear during painful experiences (58).

Some studies have assessed the role of pain catastrophising in severity of pain related to MS, using a variety of instruments. Significant relationships between catastrophising, and pain severity, have been described (57, 59).

A recent qualitative study carrying out in-depth interviews of 25 people with MS who suffered from pain (60) found that two pain management themes were most often described by participants – one relating to pain reduction, and the other to acceptance. The authors suggested that pain catastrophizing, acceptance and endurance could be treatment targets (57).

1.1.4.1.9.2 Executive Functions

Executive functions have been defined as “those capacities that enable a person to engage successfully in independent, purposive, self-serving behaviour” (61). More general definitions include supervisory cognitive processes, which involve higher level organization, and execution of complex thoughts and behaviour (62)

Specific executive functions might include initiation, planning, purposive action, self-monitoring, self-regulation, volition, inhibition and cognitive flexibility (set-shifting) (63). Executive functions have most often been localised to the frontal lobes. Many tests of executive function are felt to be sensitive, but not specific, markers of frontal lobe damage (62). Both frontal and non-frontal regions are likely to be necessary for intact executive functions, with involvement of frontal lobes likely to be necessary, but not sufficient (62). For these reasons the commonly –used term “frontal” functions is not used in this thesis.

There is some evidence for “fractionation” of executive functions (63, 64), with specific processes related to specific subdivisions of the frontal cortex.

Neuropsychological assessments, as described in this thesis, aim to measure specific processes, however it should be noted that many tasks will measure more than one at one time (63, 64).

Executive functions, in particular cognitive flexibility or “set-shifting” are thought to be important in the descending modulation of pain (33, 65). These have not however been assessed in relation to pain in MS.

1.1.4.1.10 *Cortical localisation of tests of cognitive and executive functions discussed in this thesis*

The known neuroanatomical structures thought to be relevant to undertaking selected cognitive tasks detailed in the neuropsychological battery for this study have been briefly detailed (see also Table 2 for summary of cortical localisation, and Table 15 for a summary of relationships between study instruments, and functions assessed).

1.1.4.1.10.1 Phonemic verbal Fluency

In lesion and clinical studies, phonemic verbal fluency has been demonstrated to be related to left frontal lobe function (62), in particular dorsolateral prefrontal cortex function as well as other frontal areas including ventrolateral prefrontal cortex, dorsomedial frontal cortex (63) anterior cingulate and frontal pole (64). Right-sided, and non-frontal regions have also been implicated by some studies (62).

Neuroimaging studies have suggested that phonemic verbal fluency is mainly associated with left dorsolateral prefrontal cortex activation, but also other areas including ventrolateral prefrontal cortex, thalamus, temporal and parietal cortices, and anterior cingulate (62, 64).

1.1.4.1.10.2 Hayling sentence completion

In patient and lesion studies, performance in the Hayling Sentence Completion Task has been linked to the Anterior Cingulate Cortex, as well as (less often) orbitofrontal cortex and frontal pole (64). In neuroimaging studies, completion of the Hayling test has been linked to left lateral prefrontal cortex (including inferior and dorsolateral cortex) as well as medial superior frontal cortex including anterior cingulate (64).

1.1.4.1.10.3 Reverse digit span

Patient and lesion studies have suggested that damage to dorsolateral prefrontal cortex adversely affects performance on reverse digit span (64). Neuroimaging studies have consistently pointed to the significance of DLPFC in performance of the reverse digit span, though other areas including Anterior Cingulate, Frontal Pole, Orbitofrontal cortex have been implicated (64).

1.1.4.1.10.4 Number:letter sequencing

Limited patient and lesion studies have supported the role of dorsolateral prefrontal cortex in the Trail-making test, as well as anterior cingulate in one case (64). Neuroimaging studies have similarly supported the role of dorsolateral prefrontal cortex, and in some studies anterior cingulate (64).

1.1.4.1.10.5 Summary table

This data (Table 2) is based on review by MacPherson and colleagues (64) as well as articles by Stuss (63), and Alvarez and colleagues (62). For each test/group of tests the following are separately listed: “clinical or lesional studies” which typically assess the structural imaging correlates of a relevant CNS injury or disease process, and “imaging” studies which typically use fMRI to assess functional correlates of these tests.

The symbols “++” and “+” denote my own interpretation of the information provided with regards to the strength or repeatability of an observed association.

Table 2: Cortical localisation of functions tested in neuropsychological assessments

		Cortical localisation					
		DLPFC	ACC	POLE	OFC	VLPFC	OTHER
Phonemic Fluency	Clin	++	+	+		+	
	Img	++	+			+	Temporal, parietal, thalamus
DKEFS	Clin	++	+	+			
	Img	++					Occipital, temporal, parietal, widespread
Hayling	Clin		++	+	+		
	Img	++	+				
Reverse Digit Span	Clin	++					
	Img	++	+	+	+		
Numb:letter sequence	Clin	+	+				
	Img	++	+				
++ = strong association, frequently reported + = often reported Clin = clinical or lesional studies Img = neuroimaging studies DKEFS = Delis Kaplan Executive Function System DLPFC = Dorsolateral Prefrontal Cortex ACC = Anterior Cingulate Cortex POLE = Frontal pole OFC = Orbitofrontal Cortex VLPFC = Ventrolateral Prefrontal Cortex							

1.1.4.1.11 *Sensation*

A variety of studies have analysed sensory involvement in MS patients with neuropathic pain. Methods used have ranged from standard neurological examination (15, 66) to the use of quantitative sensory testing (QST) and other techniques (26, 67-69). Substantial variation between patients with regard to degree and modality of abnormalities has been noted (69) along with discrepancy between bedside and QST testing (15).

Some studies (15, 26) relate no difference between subjects with and without pain, while others (67, 69) identify a difference. One frequent theme in several studies has been of increased sensitivity to sensory stimuli in those with pain.

Svendsen and colleagues compared 50 people with MS and pain, 50 people with MS without pain, and 50 healthy controls. Relapsing remitting MS patients accounted for 14/50 (28%) of the MS pain group, and 28/50 (56%) of the MS control group. Cold allodynia (using acetone) was present in 9/50 pain subjects, but none of the control subjects ($p=0.003$). Temporal summation of stimuli during repetitive stimulation with a nylon filament (increasing pain scores with repeated stimuli) was present in 10 of the pain group, and 3 of the control group ($p=0.03$). Allodynia to touch (using cotton wool) was present in 4/50 pain subjects, and 0/50 control subjects (15). No healthy subjects reported evoked pain or temporal summation.

Osterberg and Boivie (69) aimed to compare sensory abnormalities in people with MS and compared a group with central pain with a group without central pain, using a range of tests including bedside sensory testing and formal QST. 32% of the group of 62 people with MS and central pain had RRMS. Importantly, the method for diagnosing central pain was not stated, though subjects undertook a questionnaire before participation. Almost all of the cohort with central pain had abnormal sensation in the area of pain, most often described as abnormal sensation to temperature or pain. Allodynia (to touch, cold or pinprick) was found in 10 of 62 people with central pain, and 6 of 16 controls. The results were interpreted as suggesting spinothalamic dysfunction in MS central pain.

Truini and colleagues (70) studied 10 patients with ongoing neuropathic extremity pain selected from a sample of 300 consecutive clinic attenders with MS defined by McDonald criteria. Patients with extremity pain predominantly described a burning pain predominantly in the lower limbs. They were compared to 18 patients from the same group describing Lhermitte's phenomenon. The authors used laser evoked potentials (LEPs) as well somatosensory evoked potentials, and found that LEPs were more frequently abnormal in the extremity pain group, than the Lhermitte's group. The opposite was found to be true in the Lhermitte's group. All had thermal pain sensory deficits though the presented results do not mention presence of hypersensitivity or allodynia in any case.

Taken together, these studies suggest that there may be increased sensitivity to sensory stimuli in people with MS-associated pain syndromes. Pain syndromes in these studies are not always well-defined, and may be heterogeneous. These findings could suggest deficits in descending pain modulatory systems in people with pain in MS, though spinothalamic dysfunction has also been suggested.

1.1.5 Neuroimaging of pain in multiple sclerosis

Fundamentals of magnetic resonance imaging

I have given a very brief overview of the physical basis of MRI signal, in order to provide context for discussion of specific techniques elsewhere. I have based this brief discussion on lecture notes produced by the University of Edinburgh “Neuroimaging for Research” distance learning MSc (www.ed.ac.uk/clinical-sciences/edinburgh-imaging/education-teaching/degree-programmes/neuroimaging-for-research-msc-dip-cert) as well as published material (71-74).

Magnetic resonance imaging (originally termed nuclear magnetic resonance) relies on the physical properties of an atom’s nucleus, in particular the nuclei of hydrogen atoms contained within water, which is found throughout human body tissues in vivo. In contrast to X-Ray and CT imaging, it does not require the use of ionizing radiation, which may be associated with health concern.

When a subject lies within an MRI scanner, the single proton which makes up the hydrogen nucleus will align parallel to the axis of the scanner’s magnetic field (the principal magnetic field of the scanner is referred to as B_0 , and the axis of this field as the z axis). Some protons will align in the same direction as B_0 , and some in the opposite direction. The net magnetization of proton magnetic fields along with B_0 is referred to as M_0 (71).

Delivery of a brief current to the head coil can be used to create a radiofrequency pulse which will “tip” M_0 away from the z axis. Subsequent realignment of protons along the z axis leads to emission of a radiofrequency pulse, which is detected by the head coil.

The time taken for net proton magnetization to reach around 63% of M_0 is referred to as “T1”. T1-weighted images can be acquired by early measurement of proton magnetization intensity. T1 images are conventionally used for structural MRI brain imaging, and show grey matter as darker than white matter, CSF as dark, and fat as bright. T2-weighted imaging relies on rotation of protons after administration of the radiofrequency pulse. The time taken for the net proton magnetization to reach 37% of its original value after administration of the RF pulse is termed T2. On T2

weighted imaging, white matter is darker than grey matter, CSF is bright, and fat is bright.

FLAIR (Fluid Attenuated Inversion Recovery) imaging relies on acquiring data when CSF magnetization is zero, and has the effect of rendering CSF black in the relevant image. This enables clearer identification of parenchymal hyperintensities, particularly in areas adjacent to CSF boundaries where hyperintensity might be obscured by adjacent high CSF signal on standard T2 imaging.

Fundamentals of functional MRI imaging

Imaging techniques used for assessing human brain function in vivo include SPECT (single-photon emission computed tomography), PET (positron emission tomography) and fMRI (functional MRI). Spatial resolution of fMRI is typically in the order of 2-3 mm, whereas PET is typically in the order of 3-4 mm, and SPECT around 7-8mm. fMRI is able to resolve events of less than 1s duration, whereas PET and SPECT give temporal resolutions of tens of seconds (71). Therefore fMRI has relatively favourable spatial resolution, but particularly temporal resolution. FMRI is widely employed as a noninvasive and safe experimental technique, which allows exploration of human brain function in vivo (75).

Functional MRI relies on neuronal metabolism and attendant blood flow changes, and measures BOLD (“Blood Oxygen Level Dependent”) signal. fMRI was first described in 1990 in rats (74). This technique relies on physiology of local intracerebral blood supply.

The brain makes up only around 5% of body weight, but uses around 20% of the body’s energy supply. Energy supplied to the brain is almost exclusively in the form of glucose delivered through the blood supply. Magnetic resonance imaging is very sensitive to the differing magnetic properties of oxyhaemoglobin and deoxyhaemoglobin.

Increased local neuronal activity is thought to lead to increased blood supply to that region. The increase in blood flow is greater than that needed to meet the demands of neuronal tissue for oxygen. Therefore there a relative surplus of local blood oxygen

arises, and the relative amount of oxyhaemoglobin present locally increases. The resultant change in MRI signal can be measured as the BOLD signal, and is the basis for most fMRI experiments (71, 75)

In relation to cognitive tasks, BOLD signal is generally felt to reflect the neural responses elicited by a stimulus, and BOLD signal changes are linearly related to neural responses. BOLD responses have been found to be most correlated with local field potentials, which could imply that local BOLD signal is most related to input and processing in the relevant area (72, 73).

Magnetic Resonance Imaging in multiple sclerosis

Magnetic Resonance Imaging (MRI) plays a key clinical and research role in the study of MS. From a diagnostic perspective, MRI measures are critical in the diagnosis of multiple sclerosis (76). They are also widely used in the clinical assessment of related disorders such as clinically isolated syndromes of demyelination, and neuromyelitis optica. From a research perspective, MRI is widely used in studies of multiple sclerosis to quantify both Central Nervous System (CNS) demyelination and atrophy in vivo. MRI measures of demyelination and atrophy are widely reported in studies of multiple sclerosis treatments, and are increasingly reported as surrogate outcome measures in clinical trials (77, 78).

Separately, MRI is extensively used in the study of CNS structure and function in patient suffering from various clinical pain syndromes, as well as in healthy volunteers in studies of experimental pain conditions (1, 79, 80). Other functional imaging techniques (particularly positron-emission tomography – PET, but also single-photon emission-computed tomography -SPECT) are also widely used in the study of pain neurobiology in humans (81).

Despite the central importance of neuroimaging both in the study of MS, and of pain mechanisms, however, the use of neuroimaging to study pain syndromes associated with MS remains a developing field. Improved understanding of the neuroimaging correlates of pain syndromes in MS could guide clinicians in identifying, classifying and treating specific MS pain syndromes.

Neuroimaging measures could also hold potential to guide development of pain treatments in the future. While studies of disease modifying therapies in MS commonly employ neuroimaging outcome measures (82, 83), the relationships of imaging findings to the development, maintenance or severity of pain syndromes is unknown.

Structural MRI brain imaging of pain and related phenotypes

This introduction aims to summarise relevant data and themes regarding the structural imaging of adults with pain syndromes. A brief discussion of selected concepts closely related to pain neuroimaging, for instance neuroimaging in MS, and neuroimaging of psychiatric disorders relevant to this thesis – in particular depression, is included.

Structural MRI brain imaging of the MS disease process

The imaging investigation of focal MS lesions, and regional grey matter volumes in people with MS, has been briefly reviewed.

In imaging studies of people with multiple sclerosis, perhaps the most common abnormality investigated are focal lesions. Many studies also assess grey matter volumes. Further studies assess other imaging correlates of the MS disease process using a variety of more complex techniques (see below). In relapsing remitting MS, disease activity is detected between 5 and 10 times more frequently on conventional MRI, than clinically (on assessment for relapses). The correlation between T2 lesion measures and disability is often, however, limited (84).

1.1.5.1.1 *Imaging of MS lesions*

1.1.5.1.1.1 Typical shape and location of lesions

MS lesions are typically round or ovoid in shape. Size may range from a few millimetres to more than one centimetre. Lesions tend to be preferentially located in the periventricular white matter, brainstem and cerebellum (78).

1.1.5.1.1.2 Imaging and pathological heterogeneity of MS lesions

Data from pathology studies and advanced in-vivo neuroimaging suggest that MS lesions are not identical at the microscopic level.

Ex-vivo studies suggest that active MS lesions are typically infiltrated by macrophages, and sometimes multinucleated astrocytes termed Creutzfeld-Peters cells (78), though there is marked pathological heterogeneity. Lesions may be found to include inflammatory, demyelinating, gliotic changes or axonal injury, as well as remyelination. However imaging appearances using standard sequences are non-specific. It has been pointed out that many autopsy or biopsy data may necessarily be based on atypical cases, thus introducing potential bias (78).

T2 hyperintense lesions, which appear radiologically relatively homogeneous, can be shown to be radiologically heterogeneous by use of “advanced” imaging techniques such as magnetization transfer ratio (MTR) imaging and spectroscopy (84).

Differentiation between acute and chronic lesions can be difficult and is typically assessed by administration of Gadolinium contrast (which is used to assess breakdown of the blood-brain barrier). Contrast enhancement may persist for approximately 2-6 weeks (78).

1.1.5.1.1.3 Normal-appearing white matter (NAWM)

This term is used to refer to white matter tracts which appear normal on conventional structural MRI imaging, but pathologically (and/or using advanced imaging methods) may be found to manifest abnormalities which at a cellular level include gliosis, demyelination and infiltration by macrophages and round cells. Axonal density is also typically reduced (78).

Tract abnormalities in NAWM have been found to overlap only to some extent with T2 lesion location, suggesting that mechanisms of NAWM damage operate at least partly independently of T2 lesion location (85). Although the structural assessment of NAWM is not included in this study, it is relevant to note that apparently normal white matter may harbour pathologically and clinically significant abnormalities.

1.1.5.1.1.4 Grey matter lesions

Specific imaging sequences such as double inversion recovery, and phase sensitive inversion recovery, have been developed to increase sensitivity of MRI imaging to cortical lesions (86). These methods are not however widely used for reasons which

include availability of imaging sequences, inter-observer agreement, and vulnerability to imaging artefacts (85). Magnetization Transfer Ratio (MTR) imaging is often used to explore grey matter integrity along with other techniques such as diffusion tensor imaging, and spectroscopy (85).

1.1.5.1.2 *Imaging of grey matter atrophy in MS*

Marked atrophy of the brain is frequently reported in imaging studies of MS.

Specifically, local and overall brain volume is often significantly reduced compared to healthy controls without MS. This is particularly the case in progressive phases of MS (78). Estimates have suggested that in healthy subjects, brain atrophy will occur at a rate of between 0.1 and 0.3% per year, whereas in people with MS, this rate will be considerably higher at 0.5% to 1% per year (84). Frontal, temporal and parietal regions may be the most often affected by atrophy. Grey matter atrophy can occur even in early disease (78).

1.1.5.1.2.1 Possible substrates of imaging findings of atrophy

Neuronal and glial loss have been associated with grey matter atrophy, and have been suggested as a likely substrate (78). Cortical atrophy has been associated with severe reduction in synaptic density. This in turn may suggest atrophy of nerve cell processes, and an impairment of inter-neuronal connectivity (78). Outside the context of MS, grey matter volume loss has been related to be associated with neuronal loss and reduced cell density, or alternatively changes in glia or cell volume. Other evidence points towards reduction in synaptic density (87).

1.1.5.1.3 *Structural MRI in people with comorbid MS and depression*

One older study (2004) described the comparison of relatively small groups of people with MS, with (n=21) and without (n=19) major depression by DSM-IV (88) definitions (89). 1.5Tesla MRI brain imaging was used, and lesions were segmented using a semi-automated intensity-based technique in Analyze. Regional lesion distribution relied on compartmentalisation using landmarks in the Talarach atlas. There were no between-group differences for any of the cognitive assessments from the Rao Brief Repeatable Battery (90). There was no between-group difference for overall lesion load, total white matter or grey matter volumes. Depressed patients were said to have more extensive hyper- and hypo-intense lesions in the left medial

inferior frontal region, and measures of atrophy in the left anterior temporal region (89).

A recent large study from the MAGNIMS collaboration used lesion distribution mapping and VBM to examine grey matter morphology and lesion distribution contributions to the presence and severity of fatigue and depression (91). 123 MS patients (69 of whom were depressed and 54 not depressed) and 90 healthy controls were included (total n=213). 3.0 Tesla brain MRI imaging was used. VBM analysis was carried out in SPM 8. Lesions were segmented using Jim. Regional GM volumes did not differ between depressed and non-depressed, or between fatigued and non-fatigued patients with MS. T2 and T1 focal lesion volume or distribution did not vary according to presence of depression or fatigue.

Atrophy of the left middle frontal gyrus and right inferior frontal gyrus were related to the severity of depression (rather than presence of depression), after controlling for fatigue. These regions include the dorsolateral prefrontal cortices, and have been reported to be involved in depression in previous studies of people with depression, not comorbid with MS. The authors concluded that the structural correlates of depression in MS were not striking, but might be comparable to those in people without MS (91).

1.1.5.1.4 *Structural MRI in cognitive dysfunction associated with MS*

MRI has also been widely used in the assessment of cognitive dysfunction associated with MS. Several authors describe that particular cognitive domains are differentially affected in MS. These include information processing speed, episodic memory, and less frequently executive functions (including verbal fluency and word list generation)(85).

Many studies have reported an association between the volume and/or location of T2 hyperintense and T2 hypointense lesions with cognitive performance, both overall and in specific tests. A disconnection syndrome has been suggested, such that focal MS lesions disrupt key tracts which are core to information processing (85).

Many authors comment, however, that cognitive impairment correlates more closely with measures of grey matter volume loss than with T2 hyperintense lesions. Cortical volumes have been reported to be significantly lower in cognitively impaired people with RRMS, than cognitively “preserved” groups (84). In cognitive and other presentations, however, the relationship between MRI findings and clinical correlates is often modest (84). This has been termed the clinico-radiological paradox.

One recent study of cognitively impaired and cognitively intact people with MS used voxel based morphometry, lesion probability mapping, and diffusion tensor imaging (DTI) to explore structural and microstructural differences between the groups (92). The authors included 35 people with MS without cognitive impairment, 20 with cognitive impairment, and 30 healthy controls (total n=85). Symptoms of anxiety, depression and fatigue were considered as nuisance variables. Cognitive assessments included verbal memory and learning (using a Dutch language version of the CVLT), information processing speed (measured by an adaptation of the SDMT), spatial memory (assessed with the location learning test) and working memory (assessed with the digit span of the WAIS). Semantic memory (word fluency) was also tested. Patients were defined as cognitively impaired when their score on at least two of five neuropsychological tests fell more than 2 standard deviations below the scores of healthy controls.

No difference between cognitively impaired and cognitively intact participants was found for measures of regional grey matter volume or lesion distribution. The groups did however differ on microstructural WM tract integrity as measured by DTI (with more severe white matter integrity changes seen in the cognitively impaired group, particularly in the corpus callosum, corticospinal tracts, forceps major, cingulum and fornices (92).

A further multi-centre study from the MAGNIMS group measured cortical thinning using FreeSurfer in 60 people with RRMS, and 65 healthy controls (total n=125) (93). Tests included the Brief Repeatable Battery (90) and Wisconsin Card Sorting Test (94). Of the RRMS subjects, twenty were classified as cognitively impaired, as they scored more than 2 standard deviations below a normative value, for at least two

tests. The most common cognitive deficits were in attention, visual memory and verbal memory.

Global cortical thickness did not differ between cognitively impaired and intact subjects. Using a lobar analysis, there was a marginally statistically significant decrease in temporal lobe thickness in the cognitively intact group ($p=0.050$) with a trend to significance in the cingulate cortex ($p=0.055$). Using vertex-wise analysis, no difference was seen between cognitively intact and impaired subjects, and no correlations between cortical thickness and global cognitive scores, disability or disease duration. In analysis of cognitive subscores, verbal memory scores correlated to insular cortical thinning, whereas visual memory scores correlated to decreased thickness in parietal cortex. These recent studies may suggest that neuroimaging differences between cognitively impaired and intact subjects with RRMS are not marked.

1.1.5.1.5 *Structural brain MRI in pain syndromes outside MS*

The current literature contains many reports of differences in brain structure, assessed by MRI, between people with and without chronic pain disorders. These studies aim to further understanding of the mechanisms of central nervous system representation and processing of chronic pain.

The large majority of these studies (in contradistinction to the study described elsewhere in this thesis) include subjects with brains which appear normal to conventional MRI imaging. These subjects include people with fibromyalgia, irritable bowel syndrome, low back pain, migraine, temporomandibular joint dysfunction, vulvar pain, phantom limb pain, arthritis, menstrual pain and complex regional pain syndrome (79, 87, 95). Often these subjects are compared with healthy volunteers in a cross-sectional study design, aiming to assess central nervous system structural correlates of chronic pain syndromes.

Study designs vary in relation to the pain phenotype studied, specifics of the imaging techniques and analysis techniques used, and findings. There are however certain common themes. There is a particular focus on studies which assess grey matter volume and distribution.

Volumetric differences in a range of cortical and subcortical structures have been reported in groups with chronic pain, compared to healthy volunteers. Structures such as prefrontal cortex, insula, anterior cingulate and mid cingulate cortex are commonly reported to show volume differences in this comparison. Other regions which have been reported to be similarly affected are thalamus, basal ganglia, sensory cortex (often referred to as “S1”), sensory association cortex (“S2”) and brainstem. In addition, some studies have reported abnormalities in the temporal lobe and posterior cingulum (79, 87). Volumes have often been reported to be decreased in pain modulation regions (possibly suggesting dysfunctional pain modulatory systems) and increased in sensory/nociceptive areas (including sensory cortex) (moayed79, 87, 96).

The temporal association of grey matter abnormalities and pain syndromes is often unclear, although evidence of decreasing grey matter volume in association with pain

duration (in cross-sectional studies) has been interpreted as suggesting a possible causal relationship. Some authors hypothesise that the presence of chronic pain (and related variables) could be causally related to grey matter volume loss (96, 97). Longitudinal studies (80, 87) have lent support to this hypothesis and have reported reversibility of grey matter volume loss in some instances after resolution of pain syndromes (for instance an operation for hip pain due to osteoarthritis (80)).

However, potential confounding influences, even in longitudinal studies, include use of analgesics, physical activity, and concomitant affective disturbances. Such studies may however indicate that grey matter volume loss is at least partly secondary to the experience of chronic pain (79).

1.1.5.1.6 Two comparable studies of similar pain syndromes

Two studies of similar pain syndromes have been briefly reviewed, in order to illustrate differences and common themes in methodology and findings.

Younger and colleagues' study of 15 women with myofascial temporomandibular pain in comparison with 15 age- and gender-matched healthy controls used 3 Tesla 1mm isotropic T1 MRI brain data in a Voxel Based Morphometry (VBM) study. Analysis was implemented in SPM version 8 (97). Relative to healthy controls, patients were found to have higher grey matter volumes in a variety of regions including inferior frontal gyrus, anterior insula, thalamus, brainstem trigeminal nuclei and cerebellar peduncles. Lower volume was only seen in the right primary somatosensory cortex. Severity of pain was negatively correlated with grey matter volume in the anterior cingulate cortex and posterior cingulate/precuneus (97). No positive associations of grey matter volumes with pain severity were found. On an examination of pain sensitivity (defined using pressure algometry) greater grey matter volumes in the trigeminal nucleus regions were positively associated with pain tolerance (ie inversely related to pain sensitivity) (97).

Moayedi and colleagues' study of 17 females with temporomandibular pain and 17 healthy females used 3 tesla MRI brain imaging (0.94 x 0.94 x 1.5mm voxels), included analysis for cortical thickness using FreeSurfer, and subcortical volume using VBM in SPM version 5 (96). The authors state that their definition of

temporomandibular pain was not as narrow as that used by Younger and colleagues. The authors used masks of a sensorimotor/pain network (comprising S1, S2, motor association cortex and mid cingulate cortex - MCC) and a cognitive/modulatory mask (comprising orbitofrontal cortex, prefrontal cortex, insula, anterior cingulate and mid cingulate). Patients were found to have lower cortical thickness in S1, frontal pole, and ventrolateral prefrontal cortex. Similar to comparable work discussed above (97), pain severity was negatively correlated with grey matter thickness in the anterior-mid cingulate (whereas Younger and colleagues described an association of pain severity with slightly more anterior cortex). A decrease in grey matter thickness in the ventrolateral aspect of the motor cortex was also found. No association of subcortical volumes with either the presence or severity of pain was found (96).

These two studies of comparable disorders illustrate the overlapping yet different techniques used, with comparable yet separable results. In common with the majority of the published literature, study subjects were compared with healthy volunteers, and brain structure was normal on conventional structural MRI imaging. In comparison to the structural imaging studies of cognition and fatigue in MS briefly described above (92, 93), sample sizes are much smaller.

1.1.5.1.7 Structural MRI in depressed subjects without *MS*

Because of the association of depression with chronic pain, literature assessing structural imaging correlates of depression has been reviewed.

A large recent meta-analysis assessed existing VBM studies of major depressive disorder and bipolar disorder (98). 50 studies including 4101 individuals were identified for major depression (of which statistical maps were available for 9, with a total of 1736 patients and 2365 healthy controls). On analysis of these 9 studies, depressive disorders were associated with smaller grey-matter volumes relative to controls in the insular cortices, inferior frontal gyrus and anterior superior temporal gyrus. There was also lower volume in ventromedial prefrontal cortex, anterior cingulate, posterior cingulate, and some lateral prefrontal regions as well as in subcortical structures including left caudate, hippocampus and parahippocampal gyrus. Some of these structures can be seen to overlap with those identified in studies of pain, as discussed above.

These findings echo previous meta-analyses of structural MRI studies including that by Arnone and colleagues (99) (101 studies, using a meta-analytical approach) which have similarly identified no global difference in global brain volume measures between depressed and non-depressed individuals, but reduced volume in depressed individuals in frontal cortex, orbitofrontal cortex, cingulate, hippocampus and striatum.

1.1.6 Functional neuroimaging relevant to pain in multiple sclerosis

In this thesis, the use of resting state functional MRI (fMRI) to investigate the functional connectivity of structures key to the descending modulation of pain is described.

Resting state fMRI

Resting state functional MRI techniques aim to identify patterns in Blood Oxygen Level Dependent (BOLD) signal using imaging acquired while the subject is lying at “rest” in the MRI scanner. Specifically the acquisition does not involve administration or execution of a “task” to the experimental subject. In the context of this study, it is particularly useful in assessing the functional integration of separate structures within the brain, specifically the key nodes of the descending pain modulatory system.

Later in this thesis, a seed-based approach to assessing functional connectivity in the resting state is described (75). Briefly, this approach involves firstly identifying a “seed” region based on pre-existing hypothesis and/or existing literature. The BOLD timecourse of the voxels within this seed is then computed for the duration of the imaging acquisition. The correlation of the seed BOLD timecourse with all other voxels within the brain is then computed, and, after thresholding, provides evidence of covariance in BOLD signal (and, by inference, neuronal activity (73)) across structures.

From an analysis perspective, the timecourse of the seed region can be viewed as the independent variable in a linear model, with timecourses of other voxels viewed as dependent variables. The analysis of relationship between independent and dependent variables can then be viewed as a linear model. Other variables can be introduced into this “general linear model” (GLM), for instance to adjust for their effect (in this context the additional variable is referred to as a covariate of no interest, or nuisance covariate).

Resting state fMRI, in providing information on functional connectivity across the brain, may provide information which is complementary to structural and clinical analyses (also described in this thesis)

1.1.6.1.1 *Issues in application and interpretation of resting state fMRI*

1.1.6.1.1.1 Inferring causality

The current study assesses correlations between BOLD timecourses across the brain. It cannot infer causality and the presence of a true correlation could be present for a variety of reasons including a direct causal relationship, indirect causal relationship mediated by another structure/network, or shared influence of a common inputting region (75, 100).

1.1.6.1.1.2 Sources of extraneous signal, or noise

One prominent problem in the analysis of resting state fMRI data is the approach (or approaches) used to deal with extraneous sources of noise. These may be particularly problematic in clinical populations (as well as paediatric or elderly populations) (75, 101). For the purposes of this brief introduction, there is a particular focus on analysis techniques aiming to address these issues.

Typically, prominent sources of noise may include head movement (which can be slow or sudden, and may involve various combinations of translation and rotation), CSF signal, white matter signal and cardiorespiratory noise, which can be particularly problematic in brainstem imaging (because of nearby CSF voids and arterial pulsation). Any of these sources of noise may decrease the sensitivity of the above model to detecting correlations of neuronal BOLD activity.

Sudden head motion is particularly problematic, however, and is known to induce spurious correlations in BOLD timecourses between closely anatomically related structures (while tending to diminish correlations between more anatomically distant structures). This remains the case even after including head motion regressors in a general linear model (101, 102). Approaches to this problem may include ICA decomposition of data followed by removal of components thought to represent extraneous noise (103) (as used in the current study) (this method may be applied “manually”, or automatically after training on a dataset (104)), or “censoring” of

volumes manifesting high subject motion (often referred to as scrubbing, this technique is available in FSL as `fsl_motion_outliers`) (105).

1.1.6.1.2 *Strengths of the use of resting state fMRI in clinical populations*

Traditionally, task based paradigms have been the mainstay of functional MRI research. These designs rely intrinsically on administration of a stimulus, and therefore a subject successfully processing this stimulus, and/or executing a specific task (75, 85). In patients with MS, however, cognitive, motor, and other manifestations may interfere with ability to execute tasks during fMRI acquisition. For instance, at the most basic level, if a subject with MS asked to tap a button, there may be motor weakness. At a less obvious level, ability to comprehend or follow instructions may be differentially affected in those with MS. Resting state fMRI techniques, in avoiding the need for appreciation and/or compliance of a stimulus, may limit such problematic issues.

Functional MRI in multiple sclerosis

fMRI studies in people with MS have often shown that, in comparison with healthy controls, there is more widely distributed cortical recruitment during task fMRI. It has been proposed that this altered recruitment pattern represents cortical plasticity or functional reorganization (in other words increased cortical recruitment is required to maintain the same level of performance). These conclusions have been supported by demonstrated correlations between measures of fMRI activation pattern, and measures of brain structural damage (including lesion volume, damage to normal-appearing white matter, and grey matter damage) (79;102). As discussed, however, this conceptualisation may represent an over-simplification.

Loitfelder and colleagues (2011) studied a group of people with MS and clinically isolated syndrome (CIS) using cognitive assessments and functional MRI, in order to assess the idea of functional reorganization or plasticity in relation to cognition (106). Their cross-sectional study included 30 people with MS/CIS (10 CIS, 10 RRMS, 10 SPMS) and 28 healthy controls. Exclusion criteria included known psychiatric disorder, clinically significant depression or fatigue, and recent relapse or steroid. Functional MRI included a go/no-go discrimination test. Cognitive assessment included the Wisconsin Card Sorting Test (94) and Brief Repeatable

Battery. In fMRI analyses, higher T2 lesion volume was negatively correlated with activation in the right parahippocampal gyrus, middle frontal gyri and left putamen. Although people with MS performed worse than people without MS on the cognitive tests, performance among those with MS was similar. There was, however, evidence of more widespread BOLD signal in those with RRMS and SPMS when executing the same task (in comparison with healthy controls and CIS patients) which was interpreted as evidence of functional reorganization/plasticity.

Sbardella and colleagues (107) used resting state seed-based functional MRI as a component of multiparametric imaging and cognitive assessment of 54 people with RRMS, and 24 healthy controls. A seed based in the bilateral dentate nuclei of the cerebellum was used, in order to investigate clinical correlates of dentate nucleus functional connectivity. Patients with MS were found to manifest higher functional connectivity than healthy controls to a range of structures including frontal gyri (superior, middle and inferior), supplementary motor area and pre-central gyrus. Functional connectivity with the cerebellum, right thalamus, frontal and parieto-occipital cortices was inversely correlated with lesion volume. Functional connectivity was also inversely correlated with fractal anisotropy values from Diffusion Tensor Imaging. The authors did not comment on any positive correlation of functional connectivity with lesion volume. Within the areas where functional connectivity was higher in people with MS than in controls, functional connectivity was found to be inversely correlated with clinical impairments (higher connectivity to mid-cingulate and dorsolateral prefrontal cortex was correlated with better PASAT scores, and better EDSS scores).

Higher functional connectivity in patients in comparison with controls was interpreted as possibly representing compensatory functional reorganization, as was the inverse correlation of functional connectivity with structural damage. Structural imaging variables including T2 lesion volume and fractal anisotropy were however also found to be correlated to EDSS and cognitive performance, thus suggesting that apparent changes in functional connectivity may be among many factors associated with clinical outcomes.

More recently, Dobryakova and colleagues (108) used a novel data-driven Bayesian analysis of task fMRI data in 84 people with MS and 37 age- and gender-matched healthy controls, in order to make a detailed assessment of connectivity changes in relation to executive functions. Effective connectivity analysis was used to infer connectivity between specific structural nodes identified as key to the Stroop interference task.

While detailed discussion of this methodology is beyond the scope of this thesis, the authors did demonstrate a complex mixed picture of hyperconnectivity, loss of connections and reversal of connections in people with RRMS. Extra connections were found to be associated with performance deficits on the Stroop task in people with benign and secondary progressive MS (both of which were of long duration) but not in people with relapsing remitting MS (of shorter duration). The authors suggested that extra connections might therefore become maladaptive as time (and also disease duration and disability) increased. The authors suggested that a diverging connectivity patterns might suggest diverging compensatory mechanisms.

While this analysis technique is beyond that discussed in this thesis, this work may highlight that hypotheses of the relationship between functional connectivity and clinical outcomes are increasingly nuanced, and may not be adequately reflected by a simple model of compensatory functional reorganization. In particular, there were no correlations (in the RRMS group) between behavioural outcomes during the Stroop test (including reaction times and accuracy) and the strength of extra or reversed connections (108). A complex mixture of increased and decreased connectivity was found, with the possibility of adaptive and maladaptive changes considered.

Functional anatomy of descending pain modulatory system

Key structures of the DPMS have been discussed. Please also see above (Table 2: Cortical localisation of functions tested in neuropsychological assessments) for an overview of the involvement of these, and other, structures in cognitive and executive functions.

1.1.6.1.3 *Anterior Cingulate Cortex (ACC)*

The anterior cingulate cortex is reliably activated in fMRI studies assessing the pain condition. It has been found to be key to the descending modulation of pain, in particular the modulation of pain by attention and expectation (33, 109). It is thought to exert top-down influences to the periaqueductal gray matter in association with prefrontal cortices, in particular the dorsolateral prefrontal cortex and ventromedial prefrontal cortex. The rostral ACC in particular plays a key role in mediation of placebo responses, and in this context functional connectivity between rostral ACC (rACC) and PAG has been found to be particularly important (110) (see below).

In healthy controls, direct structural connectivity between rACC and PAG has been demonstrated using diffusion tensor imaging (111).

The ACC has also been found to be strongly involved in emotional processing, and this role may be particularly important in the interaction of emotional/affective state and the descending modulation of pain (29). It has been suggested to be important in a range of functions including cognitive modulation of pain affect and placebo analgesia, emotional-affective processing of pain (29), and attentional modulation of pain (33).

1.1.6.1.4 *Periaqueductal Grey matter (PAG)*

The periaqueductal gray matter (PAG) is a key node in the descending modulation of pain and is involved in both inhibitory and excitatory top-down regulation of pain (1, 30, 32, 110). Its anatomical and functional subdivisions are however not yet fully understood. In addition to its role in the modulation of pain, it has been found to be involved in respiration, fear, anxiety and other physiological responses.

It has also been found to play a critical role in encoding error judgements related to prediction errors, following top-down influence from the ventromedial prefrontal cortex. The “avoidance value” of pain might then be updated by PAG input to the orbitofrontal cortex and anterior midcingulate (112). There is thought to be functionally relevant subdivision of the PAG, with the ventrolateral PAG in particular being involved in opioid-mediated descending modulation of pain (113).

1.1.6.1.5 *Prefrontal cortex, especially dorsolateral prefrontal cortex*

The prefrontal cortex (and in particular the dorsolateral prefrontal cortex) has also shown to be closely related to the ACC, and may have a role in “keeping pain out of mind”, perhaps mediated through the ACC (and/or the ventrolateral prefrontal cortex) (33). The prefrontal cortices (particularly dorsolateral prefrontal cortex, but also ventromedial prefrontal cortex) are thought to impose a top-down bidirectional influence on key structures such as PAG, amygdala and anterior insula (1, 29). Key relevant cognitive roles include attention, expectations and appraisal in the experience of pain. Distraction may be part of the mechanism whereby prefrontal cortices contribute to the DPMS (33)

The prefrontal cortex has particularly been implicated in distancing the self from the experience of pain, which could be linked to alterations in learning about anxiety-related cues, selective attention, and interpretative biases (29). The ventrolateral prefrontal cortex (VLPFC) may also have an important role in the modulation of aversive stimuli based on reappraisal (33).

1.1.6.1.6 *Inter-relationships of rACC, PAG and DLPFC in investigation of descending pain modulation*

1.1.6.1.6.1 *Characterisation of PAG functional connectivity without intervention*

A recent resting state fMRI functional connectivity study assessed 79 healthy volunteers, specifically examining the differential functional connectivity of different locations within the PAG (113) with the aim of delineating functional neuroanatomy. The authors used 3Tesla acquisitions with TR of 2 seconds, and a total of 150 volumes acquired. Following statistical clustering of PAG anatomy (into a bicolumnar model) the authors described functional subspecialisation within the PAG, particularly including the ventrolateral PAG (MNI coordinates $\pm 3, -32, -12$) and dorsolateral PAG (MNI coordinates $\pm 2, -32, -5$).

The ventrolateral PAG was found to be functionally connected to rostral Anterior Cingulate Cortex, Thalamus, Pons, Cerebellum, Caudate and Putamen. Functional connectivity of the ventrolateral PAG (in contrast to other PAG subdivisions) was found to be stronger to the dorsolateral prefrontal cortex, frontal pole, various subcortical structures (thalamus, putamen and caudate) and parietal cortex, as well as

parahippocampal gyrus/hippocampus. Dorsolateral PAG was found to be functionally connected to premotor cortex, dorsolateral prefrontal cortex, caudate, putamen, thalamus and frontal pole. Stronger functional connectivity (in comparison to the ventrolateral PAG) was found to the premotor cortex, motor cortex, and supraparietal lobule.

1.1.6.1.6.2 Characterisation of PAG structural connectivity without intervention

These findings are concordant with a separate study in healthy volunteers, which used DTI imaging to assess structural (rather than functional) connectivity of the PAG. The authors used 3Tesla MRI brain imaging (including diffusion data) and imaged 19 healthy subjects. Brainstem optimisation of the imaging protocol included cardiac gating of the structural imaging, to limit the effects of CSF pulsatility. Structures involved in modulation of pain, stress and anxiety (including prefrontal cortex, hypothalamus and amygdala) were found to be dominantly connected to the ventrolateral PAG (114).

1.1.6.1.6.3 Interventional fMRI study investigating placebo response (healthy volunteers)

In an fMRI study of 48 healthy volunteers, Eippert and colleagues used the administration of a placebo analgesic, as well as naloxone, to investigate the behavioural and physiological responses to pain and their modulation by the placebo response, as well as the role of opioidergic signalling in the placebo response (which was inferred by interactions with administration of naloxone) (110). The analysis was particularly focussed on structures known to be relevant to endogenous modulation of pain (DPMS), in this case focussing on the placebo response. The authors particularly focussed on DLPFC, rostral ACC and PAG, as well as examining other structures including amygdala, hypothalamus and rostral ventromedial medulla (RVM). The findings in general supported a hypothesis that functional connectivity of these regions was important in the endogenous modulation of pain. Specifically, the individual functional coupling between rACC and PAG (ie the strength of association of BOLD timecourses of the two regions) was found to be strongly related to the strength of clinical placebo response. The strength of this coupling was also associated with RVM BOLD responses, further suggesting that

this coupling is linked to recognised DPMS pathways. The DLPFC showed significant activations under placebo compared to control conditions. Furthermore, the administration of naloxone substantially interfered with behavioural and physiological findings related to placebo response, suggesting that endogenous opioidergic signalling is key to this system.

1.1.6.1.6.4 Resting state fMRI examinations of descending pain modulation, in subjects with pain conditions

1.1.6.1.6.4.1 *Chronic back pain*

Yu and colleagues studied functional connectivity of a small PAG seed (set in the ventrolateral PAG) with other brain regions including seeds in rACC/ventromedial prefrontal cortex (ie rACC or slightly more anterior), insula and amygdala (115). They studied 18 people with chronic non-specific low back pain, and 18 healthy controls matched for age and gender. The data were acquired on a 3T Siemens scanner with 32 channel head coil, and 6 minute acquisition at TR 3000ms (between 103 and 113 volumes remained after censoring of high-motion timepoints). Subjects lay with their eyes open during the acquisition. Connectivity between PAG and rACC/vmPFC was found to be increased in subjects with pain, compared with healthy controls. This was interpreted as either an enhanced inhibition, or facilitation of pain by endogenous mechanisms. The authors also described a negative correlation between PAG:rACC/vmPFC coupling with pain severity.

1.1.6.1.6.4.2 *Migraine (interictal study)*

Mainero and colleagues (103) studied 17 people with migraine interictally, and 17 healthy controls (gender and age matched) with resting state fMRI (3T Siemens system, 32-channel coil , TR 3000ms). Bilateral PAG spherical ROIs (3mm radius) were used for the seed analysis, and the data preprocessed in a similar way to that described in the current study (including ICA denoising, but without the CSF or WM nuisance regressors used here). Functional connectivity of PAG with brain regions implicated in pain modulation (prefrontal cortex, anterior cingulate and amygdala) was found to be weaker in those with more migraine attacks. Specifically, when tested against frequency of migraine attacks, there was a negative correlation with PAG connectivity with regions including dorsomedial PFC as well as right rostral

anterior cingulate (peak coordinates $x=6$, $y=52$, $z=16$). Furthermore, decreased connectivity of PAG with structures including R dorsolateral prefrontal cortex ($x=36$, $y=50$, $z=6$) was found in those with, compared to those without, allodynia. It is worth noting that these coordinates are described as frontal pole using probabilistic atlases packaged with FSL (116), thus perhaps suggesting overlap with other frontal cortical structures known to be implicated in cognitive modulation of pain (96).

Structural imaging of cervical cord

As discussed elsewhere, the structural imaging data described in the current study is designed to acquire MRI imaging of the brain.

As a complex neuroinflammatory and neurodegenerative condition, however, multiple sclerosis affects both the brain and spinal cord (28, 117). Cervical and thoracic spinal cord imaging indices have repeatedly been strongly associated with disability in multiple sclerosis (117-119).

MRI detectable lesions of the spinal cord can be found in up to 90% of patients with definite MS. Cord atrophy is also found, and is most severe in progressive forms of the disease (84). Most white matter pathology in the spinal cord is seen in the cervical cord, whereas grey matter damage is seen more extensively throughout the cord (78).

Typically, however, spinal cord imaging studies in multiple sclerosis use tailored spinal imaging sequences including appropriate centring of the field of view, use of a specific spinal cord coil, and in the context of research studies, often a specific acquisition optimised for research studies (such as Phase Sensitive Inversion Recovery - PSIR (119)).

The research utility, if any, of upper cord data available from brain imaging studies, as in the current study, is unknown. Signal drop-out and artefact towards the edge of the scanned volume might adversely affect the utility of this data.

Acquisition of brain and spinal imaging was judged to be prohibitive for the current study in terms of patient imaging burden, particularly taking into account possible effects of increasing imaging duration on subject movement, movement artefact and

image quality. Please see Appendix: Participants' Experience (p400) for participants' own assessments of imaging duration, and comfort in the MRI scanner within this study.

One recent published study by Liu and colleagues (118) has attempted to assess the feasibility of using volumetric brain imaging data (as acquired in the current study) in a post-hoc analysis of cervical cord cross-sectional area including people with MS. One other conference abstract (120) assessing a similar technique has not yet been subject to peer review at time of writing.

Liu and colleagues (118) used a 3T Philips Achieva system, with a 32-channel head coil, and acquired volumetric 1mm³ T1-weighted brain imaging without use of additional spinal sequences or a spinal coil. Data from 13 healthy controls, and 37 people with MS (mixed phenotype, 17 RRMS and 20 progressive MS subtypes) were studied. The software package Jim was used for image analysis, implementing a seed-growing technique for cord/CSF boundary segmentation (121). Papinutto and colleagues (120) describe in their abstract, that volumetric MPRAGE sequences were acquired using a 20-channel head and neck coil. Other spinal imaging (PSIR or T2 weighted images) was also acquired in a total of 80 patients at two centres (Boston, and San Francisco). Again the proprietary software package Jim was used for analysis (121).

In the one peer-reviewed study examining this technique specifically, CC-CSA measures at different levels were found to be highly correlated with each other, and moderately-highly with ICV. Correlation with disability measured by EDSS was weak, though correlation with other clinical measures such as timed 25foot walk (not measured in the current study) was higher. Effects of gender are not reported by Liu and colleagues, however previous studies have reported higher CC-CSA in males than females (122).

The clinical and research implications of this data are as yet not fully understood. Furthermore, the utility in studies of pain have not been investigated. For this reason an exploration of the use of this data is included in the current thesis.

1.2 Overview of thesis structure

Five experimental chapters are included in this thesis.

1.2.1 Systematic Reviews

The first and second experimental chapters (Chapters Two and Three, with Chapter One the Thesis Introduction) deal with separate systematic reviews respectively addressing aspects of the epidemiology, and neuroimaging correlates, of pain syndromes in MS.

1.2.2 Prospective clinical study

The Fourth, Fifth and Sixth chapters deal with the three separate sets of data drawn from the same prospective controlled study of people with relapsing remitting MS, with and without neuropathic limb pain.

These chapters discuss

- Clinical assessments (including neuropsychological and other assessments)
- Structural MRI imaging assessment
- Functional MRI imaging assessment (in the resting state).

1.3 Aims of thesis

These aims are divided into five chapters, reflecting the experimental chapters included in this thesis.

1.3.1 Systematic Review: Prevalence, associations and natural history of pain in multiple sclerosis

Pain prevalence

Aims were to establish:

- The estimated overall prevalence of pain in people with MS
- The heterogeneity associated with this estimate
- Possible roles of study design and study sample factors associated with reported prevalence of pain in MS.
- The prevalence of common pain syndromes in MS
- The prevalence of simultaneously co-occurring pain syndromes in MS.

Pain incidence

Aims were to establish:

- Any estimates of pain incidence (defined as rates of first occurrence of any pain syndrome) in pwMS.

Natural history of pain in MS

Aims were to ascertain:

- Estimates of longitudinal variation of pain prevalence in MS throughout the disease course, using, where available:
 - Estimates at specific pre-defined time points in the disease course, and
 - Longitudinal follow up of a population in which prevalence of pain had been quantified at baseline.

Relevance to design of clinical study

The design of this systematic review, and results arising from it, are directly relevant to design of the clinical study described elsewhere in this thesis. Areas of particular relevance are outline below (Table 3).

Table 3: Planned use of systematic review data in design of clinical study

Data sought from systematic review	Relevance to design of clinical study
Overall prevalence of pain in populations with MS.	Will inform estimations of possible pool from which subjects with pain might be recruited.
Prevalence of specific pain syndromes	Will inform estimates of possible pool from which subjects with specific pain syndromes might be recruited.
Clinical associations of pain in MS	May identify potential demographic differences (if any) which might be anticipated between subjects with and without pain, in a case-control study
Variations of pain prevalence during disease course	Will inform estimations of possible pool from which subjects might be recruited, in early vs late disease, and in RRMS vs progressive disease subtypes.

1.3.2 Systematic Review: Neuroimaging correlates of pain syndromes in multiple sclerosis

In order to identify gaps in knowledge, and highlight future research priorities, this review summarises and appraises existing studies of neuroimaging correlates of MS pain (using MRI, PET or SPECT), and assesses neuroimaging evidence for aetiology of individual pain syndromes in MS.

Specifically, aims included to establish:

- Existing knowledge of the relationship between any pain syndromes, and neuroimaging findings in adults with MS
- Methodological characteristics of existing studies
 - Including type of imaging used, and study quality
- The strength of any identified associations between neuroimaging findings and pain syndromes.

Relevance to clinical study

Detailed knowledge of the existing literature will provide a basis for design of clinical and neuroimaging studies described elsewhere in this thesis.

- In particular, detailed knowledge of MS disease features seen using neuroimaging in specific pain syndromes (including focal demyelinating plaques, lesion burden and atrophy) will inform the imaging acquisitions and analysis planned for the neuroimaging study described later in this thesis.

1.3.3 Prospective experimental study: Clinical, behavioural and neuropsychological associations of neuropathic limb pain in adults with relapsing remitting multiple sclerosis

Study aims included to:

Recruit a group of people with relapsing remitting MS and well-characterised neuropathic limb pain, along with a control group of people with MS, but without a pain disorder.

Carry out targetted assessment of a range of variables known to be relevant to the descending modulation of pain in these groups, including

- Pain severity
- Medications
- Emotional/affective variables:
 - anxiety, depression, pain catastrophising, fatigue
- Cognitive variables:
 - Memory, executive functions and in particular cognitive flexibility
- Sensory:
 - hypersensitivity and allodynia

Assess other variables relevant to existing consensus and patient-led recommendations (123, 124) for studies of clinical pain disorders.

1.3.4 Prospective experimental study: Structural imaging associations of neuropathic limb pain in adults with relapsing remitting multiple sclerosis

Quantification of structural brain imaging characteristics

Study aims were to:

- Measure overall tissue class volumes and compare these in relation to presence and severity of pain
- Measure and compare volumes of T2 hyperintense lesions, both overall and specifically in the brainstem
- Measure and compare topography (three-dimensional distribution) of T2 hyperintense lesions, using distribution probability mapping
- Measure and compare local grey matter volumes, in relation to presence and severity of pain
- Measure any association of regional grey matter volumes with the duration of neuropathic pain.

Exploration of methods:

Study aims were to:

- Compare manual and automated methods of intracranial volume estimation in a subset of the study population,
 - In order to assess suitability of automated methods for use in the whole population, in further analyses
- Measure intra-rater reliability in measurement of T2 hyperintense lesion volume.
- Explore manual and automated approaches to estimating cervical cord cross-sectional area, extracted from brain imaging acquisitions in this study.

1.3.5 Prospective experimental study: Functional connectivity of the descending pain modulatory system in adults with neuropathic limb pain associated with multiple sclerosis

Aims were to:

- Examine functional connectivity between key structures identified as relevant to the descending pain modulatory system, and to clinical findings in the current study cohort.
 - Specifically, functional connectivity between rostral anterior cingulate cortex (rACC), dorsolateral prefrontal cortex (DLPFC) and periaqueductal grey matter (PAG) was examined.
- Compare functional connectivity between pain and control groups, and also to examine functional connectivity as a correlate of pain severity at time of imaging.
- Investigate the impact of T2 hyperintense lesion volume on any differential functional connectivity
- Investigate functional connectivity with a “region of no interest” structure not thought to be involved in the DPMS (in order to assess specificity of any findings to the DPMS).

1.4 Hypotheses

Hypotheses are discussed separately in relation to each experimental chapter, as well as stating overall hypotheses for the prospective clinical study.

1.4.1 Systematic Review: Prevalence, associations and natural history of pain in multiple sclerosis

Based on existing literature, the following hypotheses were made:

- Pain is highly prevalent in people with MS
- Various separate pain syndromes would be found in people with MS,
 - Often co-existing in the same individual.
- Prevalence of pain in people with MS will be higher in older populations with higher levels of disability.
 - Therefore pain would be increasingly prevalent during longitudinal follow-up studies.

1.4.2 Systematic Review: Neuroimaging correlates of pain syndromes in multiple sclerosis

Based on knowledge of the literature, the following hypotheses were made:

- The number of existing studies examining associations between neuroimaging features of MS, and occurrence of pain syndromes will be limited.
- Identified studies will focus on the role of focal demyelinating lesions in pain syndromes associated with MS
- Existing literature would most often employ structural MRI imaging
- Some studies would link location of focal CNS demyelinating lesions to specific pain syndromes.

1.4.3 Prospective experimental study: Overall

The following hypothesis was made:

In adults with relapsing remitting MS, dysfunction of the descending pain modulatory system will be associated with the presence, and severity, of neuropathic limb pain.

1.4.4 Prospective experimental study: Clinical, behavioural and neuropsychological associations of neuropathic limb pain in adults with relapsing remitting multiple sclerosis

Based on evidence reviewed above, in people with MS and neuropathic limb pain, the following hypotheses were made:

- Adverse affective and emotional symptoms will be related to the presence, and increasing severity, of neuropathic pain.
- Cognitive dysfunction, in particular reductions in cognitive flexibility relevant to prefrontal cortex function, will be associated with presence, and increasing severity, of neuropathic pain
- Sensory signs of hypersensitivity including allodynia will be associated with the presence, and increasing severity, of neuropathic pain.

1.4.5 Prospective experimental study: Structural imaging associations of neuropathic limb pain in adults with relapsing remitting multiple sclerosis

The following hypotheses were made:

- Detailed analysis of structural brain imaging will show differences (in T2 hyperintense lesion distribution, and/or localised grey matter volume) which are consistent with the overall hypothesis of impaired descending inhibition of pain, on comparison of the group who experience pain, with the group who do not.
- Similar differences will be found in association with pain severity, within the group who have neuropathic pain.
- There will be negative associations of regional grey matter volumes with increasing pain duration.
- Exploratory measurements of cervical cord cross-sectional area, from the brain imaging acquired in this study, will be possible.

Tissue class volumes

- There will be no difference in overall volume of grey matter, brain parenchymal fraction or intracranial volume in relation to presence or severity of neuropathic pain.

T2 Hyperintense lesion volume and distribution

- There will be no difference in overall volume of T2 hyperintense lesions in relation to presence or severity of neuropathic pain
- The distribution of T2 hyperintense lesions will differentially involve structures known to be relevant to the descending modulation of pain, or tracts connecting these structures, in subjects with neuropathic pain.
 - Specifically these structures include the brainstem, which is critical to key nodes of the DPMS (PAG and RVM) and their connection to the spinal cord.

Grey matter volumes

- There will be differences in local grey matter volumes detected between the groups with and without pain.
- These will specifically involve structures known to be relevant to the descending modulation of pain.
- Both increased and decreased regional grey matter volumes relative to subjects without pain will be observed.

Exploration of cross-sectional cervical cord area from MPRAGE brain data

- It will be possible to extract limited data on cross-sectional area of upper cervical cord from the available brain imaging data.
- There will be no difference in cross-sectional area of the upper cervical cord between subjects with and without pain.

1.4.6 Prospective experimental study: Functional connectivity of the descending pain modulatory system in adults with neuropathic limb pain associated with multiple sclerosis

The following hypotheses were made:

- Functional connectivity between the rostral ACC, DLPFC and PAG will vary between groups with and without neuropathic pain.
- It is most likely that functional connectivity of the DPMS will be strongest in the control group, reflecting functional integration of these structures in the descending modulation of pain.
 - As described, however (108), increased functional connectivity in the presence of a lesioned central nervous system has been repeatedly described in MS, and often attributed to compensatory functional reorganization.
- Inclusion of T2 hyperintense lesion volume as a covariate of no interest in the above analyses will reduce any effect observed.
- Functional connectivity with a “region of no interest” structure will not recapitulate any patterns of differential functional connectivity observed in DPMS structures.

Chapter 2 Prevalence, Incidence and Clinical Associations of Pain in Adults with Multiple Sclerosis: Systematic Review and Meta-Analysis

2.1 Introduction

As described in the Introduction to this thesis (page 1), understanding of the prevalence, associations and natural history of pain in multiple sclerosis is limited.

More detailed knowledge of these factors could allow better mechanistic understanding, as well as influencing the design of future clinical studies.

Experimental aims and hypotheses are discussed in detail in the Introduction (see page 50).

2.1.1 Peer-reviewed publication related to this work

Sections of this work were published as

P Foley, H Vesterinen, B Laird, E Sena, L Colvin, S Chandran, M MacLeod, M Fallon

- “Prevalence and natural history of pain in adults with multiple sclerosis: systematic review and meta-analysis”
- Pain, Volume 154 (5), 2013, p632-642

2.2 Methods

2.2.1 Literature search and selection criteria

Search Strategies

A database search strategy based upon detailed searches employed in recent Cochrane Database systematic reviews (125-129) was used to search Medline (from 1977), EMBASE (from 1974), and the Cochrane Library. Searches were carried out on 11th November 2011. Comprehensive sensitive searches for multiple sclerosis, and for pain, were combined using the Boolean operator “AND”.

A “forward search” was also carried out by using Cited Reference Search (Web of Science) to identify articles referencing identified publications (3rd January, 2012). Searches were limited only to studies of humans. Specifically, publications in languages other than English were included. In addition, reference lists were hand-searched, and authors were contacted to identify unpublished data.

2.2.1.1.1 *Update of literature search for thesis chapter*

During the preparation of this thesis chapter, the cited reference search was rerun using Cited Reference Search (Web of Science) in August 2016. The full search described in this chapter was not rerun, because the data generated by the principal literature search had already been subject to peer review, and had been published in a peer reviewed journal. The process of the initial literature search was deliberately exhaustive, and therefore time-consuming. For the purposes of updating the searches for this thesis chapter, I felt that the priority was to identify most papers published since the original searches, and to identify their contribution to the literature when viewed in the context of those already identified.

Database search strategies are shown below (Table 4):

Table 4: Database Search Strategies

PubMed	Embase	Cochrane Database
<i>Limits: Humans only</i>	<i>Limits: Humans only</i>	
<p>((("Multiple Sclerosis"[mh]) OR ("Myelitis, Transverse"[mh:noexp]) OR ("Demyelinating Diseases"[mh:noexp]) OR ("Encephalomyelitis, Acute Disseminated"[mh:noexp]) OR ("Optic Neuritis"[mh])) OR ((("multiple sclerosis"[Title/Abstract]) OR ("neuromyelitis optica"[Title/Abstract]) OR ("transverse myelitis"[Title/Abstract]) OR ("encephalomyelitis"[Title/Abstract]) OR ("devic"[Title/Abstract]) OR ("optic neuritis"[Title/Abstract]) OR ("demyelinating disease*"[Title/Abstract]) OR ("acute disseminated encephalomyelitis"[Title/Abstract])))</p> <p>AND</p> <p>(pain* or analgesi* or neuralgi* or headache* or toothache* or earache* or sciatica or causalgia* or arthralgi* or colic* or dysmenorrhoea or dysmenorrhea)</p>	<p>(pain* or analgesi* or neuralgi* or headache* or toothache* or earache* or sciatica or causalgia* or arthralgi* or colic* or dysmenorrhoea or dysmenorrhoea).mp.</p> <p>AND</p> <p>exp encephalomyelitis/ or exp demyelinating disease/ or exp multiple sclerosis/ or exp myelooptic neuropathy/ or multiple sclerosis.ab.ti. or neuromyelitis optica.ab.ti. or encephalomyelitis.ab.ti. or devic.ab.ti.</p>	<p>#1 <u>MeSH descriptor Multiple Sclerosis explode all trees</u></p> <p>#2 <u>MeSH descriptor Demyelinating Diseases, this term only</u></p> <p>#3 <u>MeSH descriptor Myelitis, Transverse, this term only</u></p> <p>#4 <u>MeSH descriptor Optic Neuritis explode all trees</u></p> <p>#5 <u>MeSH descriptor Encephalomyelitis, Acute Disseminated, this term only</u></p> <p>#6 <u>(multiple sclerosis):ti.ab,kw</u></p> <p>#7 <u>(demyelinating NEXT disease):ti.ab,kw</u></p> <p>#8 <u>(transverse NEXT myelitis):ti.ab,kw</u></p> <p>#9 <u>(neuromyelitis optica):ti.ab,kw</u></p> <p>#10 <u>(optic neuritis):ti.ab,kw</u></p> <p>#11 <u>(devic):ti.ab,kw</u></p> <p>#12 <u>(acute disseminated encephalomyelitis):ti.ab,kw</u></p> <p>#13 <u>(#1 OR #2 OR #3 OR #4 OR #5 OR #6 OR #7 OR #8 OR #9 OR #10 OR #11 OR #12)</u></p> <p>#14 <u>MeSH descriptor Pain explode all trees</u></p> <p>#15 <u>MeSH descriptor Analgesia explode all trees</u></p> <p>#16 <u>MeSH descriptor Analgesics explode all trees</u></p> <p>#17 <u>(pain* OR analgesi* OR neuralgi* OR sciatica OR headache* or colic* or toothache* OR earache* OR dysmenorrhoea OR dysmenorrhoea OR arthralgi* OR arthriti* OR neuropath*):ti.ab,kw</u></p> <p>#18 <u>(#14 OR #15 OR #16 OR #17)</u></p> <p>#19 <u>(#18 AND #13)</u></p>

Inclusion and exclusion criteria

2.2.1.1.2 *Inclusion criteria*

Studies characterising clearly defined pain in adults with definite MS were included. The diagnosis of MS was considered as definite where use of recognised contemporaneous criteria including McDonald (130), revised McDonald (131) or Poser (132) was described, or, if diagnostic criteria were not specified, where the diagnosis was explicitly confirmed by a neurologist (21, 44, 133, 134). The latter provision (diagnosis by a neurologist) was included in order to avoid inappropriate exclusion of rare studies where the diagnosis was confirmed by a neurologist, although specific diagnostic criteria were not stated.

Because it was hypothesised that retrospective and prospective studies of pain prevalence might differ in pain prevalence estimates, and that prospective estimates would be less prone to recall bias, only prospective studies were included.

2.2.1.1.3 *Exclusion criteria*

Studies investigating pain attributed solely to a treatment or intervention were excluded (because our experimental aims concerned pain related to the disease itself). Other study types excluded were those where subjects included in the study sample were selected for symptoms including pain (because sample selection for symptoms in this way would render calculation of pain prevalence in a sample problematic), those reporting insufficient data to calculate pain incidence or prevalence, studies of childhood onset MS (because of possible epidemiological differences from MS with adult onset (135)), and re-published data. Where interventional trials described the presence of pain, only baseline data was assessed (in order to eliminate possible effects of trial interventions).

Process of review by researchers

Titles and abstracts of all identified studies were reviewed. Potentially relevant articles published in English were then independently reviewed in full by two researchers (PF, BL) using a standardised data extraction form compiled by PF. Disagreements were resolved by consensus.

Studies published in languages other than English were reviewed by fluent medically qualified volunteers. Volunteers were identified by word of mouth, and by email requests to the University of Edinburgh Clinical Neurosciences department. No payments were made for volunteers' time. Of ten volunteers who assisted with study translation, all were medically qualified, and fluent in the language of interest. Nine of ten were specifically qualified in, or training in, clinical neurosciences or closely related disciplines.

2.2.2 Data Extraction and Analysis

Data extraction

Methodological data was extracted including; pain types studied and excluded and assessment instruments used. The timeframe over which pain was assessed in relation to the study time point (termed "pain timeframe") was recorded. For example some studies recorded pain within the week prior to subject interview, whereas some recorded pain in the month (or longer) prior to interview.

Furthermore, the following data were recorded: demographic properties of the sample, the prevalence of pain overall, and, where available, prevalence of pain syndromes, including prevalence of "neuropathic" or "somatic" pain syndromes (after O'Connor and colleagues) (5) as reported by investigators. Investigators' estimates of prevalence of co-occurring pain syndromes (ie clinically separate pain syndromes coexisting in a single individual) were also noted.

Investigators' estimates of likely pain aetiology were used because data leading to investigators' estimates (such as questionnaire results, clinical examination or clinical investigation results, or clinical interview) were not normally available to the

review's authors, and were not homogeneous between studies. Pain syndromes were selected according to availability of data, and clinical relevance. Headache subtypes could not be analysed because of overlapping groups. (136)

Quality Assessment of included studies

Quality assessment was carried out according to four criteria. These criteria were identified as being relevant to our experimental aims as described above. The following were noted: investigator blinding of any type (for instance clinical assessment blinded to pain status, which we hypothesised might reduce assessment bias on the part of the assessing team), use of, or reference to, externally available validated instruments (relevant to prevalence estimation, and in particular comparison of results with those of other studies), presence of control groups (relevant to comparison of patients with multiple sclerosis to the general population), and description of longitudinal follow-up (relevant to longitudinal characterisation respectively).

Meta-analytical and statistical analysis

95% confidence intervals of proportions were calculated by the Clopper-Pearson method, an "exact" method deriving the confidence interval from the binomial distribution. (1;2) Estimating the 95% confidence interval of a proportion by approximation to the normal distribution was judged to be inappropriate given the low number of outcomes reported in some study estimates. (137) Pooled proportions were calculated by DerSimonian and Laird random effects meta-analysis. (138) Where study numbers allowed, pooled proportions were stratified by pain timeframe into studies examining pain within one month prior to assessment, and studies examining pain over longer periods. The threshold of one month was chosen to balance study numbers in each stratum. The I^2 statistic (139) was used to estimate heterogeneity. Funnel plots were created and visually inspected, Egger and Begg-Mazumdar tests were used to estimate risk of publication bias.

Meta-regression was used to explore study and demographic variables which might influence estimate heterogeneity. Meta-regression was used because individual patient data, although preferred for this application (140) were not available. Study numbers were judged to be sufficient to allow meta-regression for estimates of overall pain prevalence, and estimates of headache prevalence. Study numbers were insufficient to allow meta-regression for other pain syndromes.

Specific methodological characteristics of studies were selected (investigator blinding, outpatient population studied and pain timeframe used); as well as demographic characteristics of the sample (mean EDSS, proportion female, proportion progressive MS, and mean disease duration) as independent variables based on availability of data, and on previously reported associations. (5)

A distinction was not made between primary progressive and secondary progressive MS (141) in the primary analysis given low numbers of studies using this classification. Given limited study numbers, univariate analyses rather than multivariate analyses were used. The significance threshold was set at the 5% level. We used a Bonferroni correction of significance level for multiple comparisons.

Relationships between pain prevalence or incidence and the MS disease course were also studied using estimates at disease milestones (prior to disease onset, at disease onset, and at relapse), and longitudinal cohort studies of overall pain. STATA v10 (meta-regression) and StatsDirect v2.7.8b (proportion meta-analysis) were used.

2.2.2.1.1 Contributions to analysis

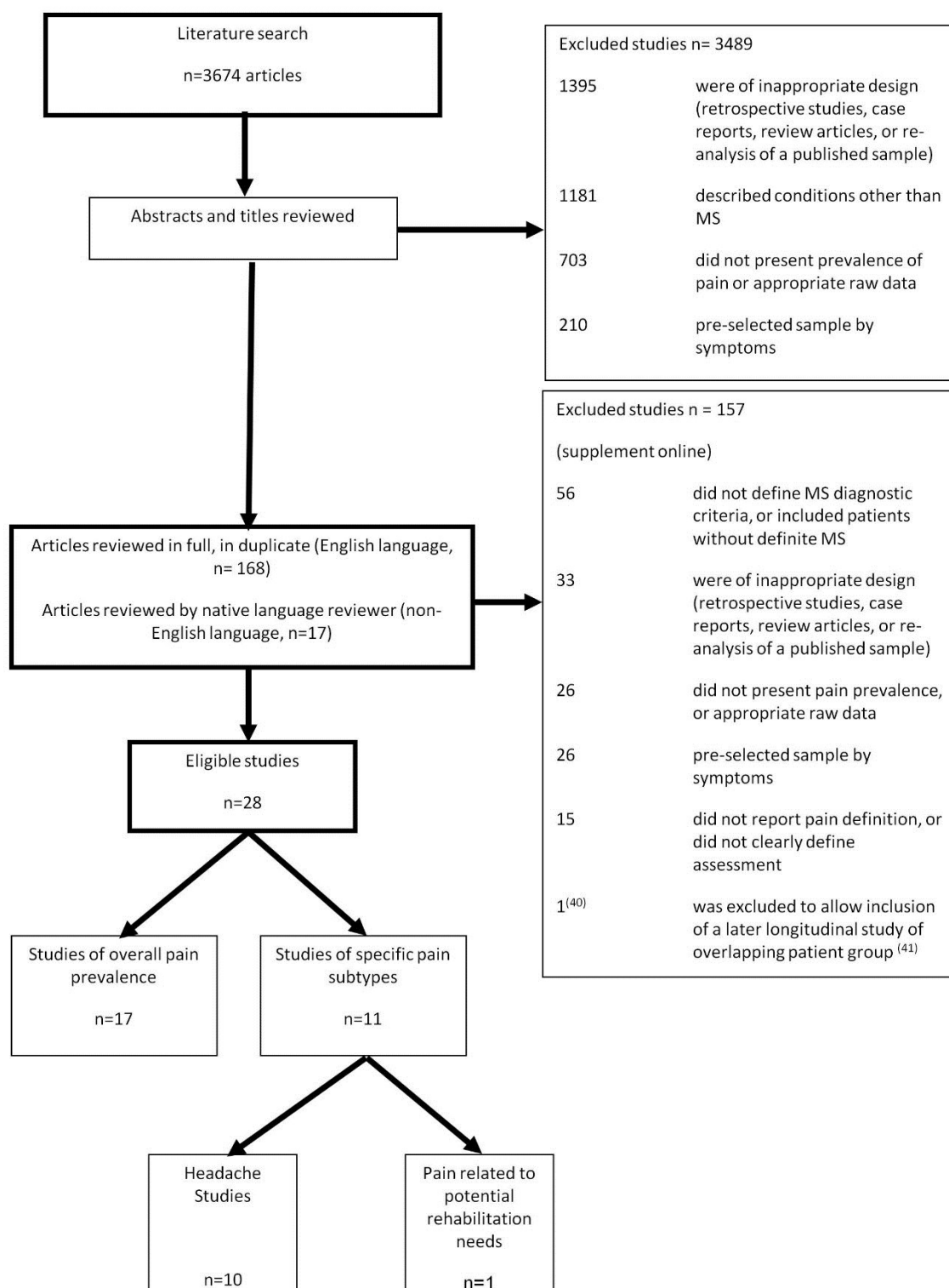
Meta-regression was carried out by Hanna M Vesterinen. Other meta-analysis was carried out by Peter Foley.

2.3 Results

Seventeen estimates of overall pain, (7, 11, 20-27, 44, 66, 142-146) and seventeen estimates of overall headache (11, 22, 24, 27, 44, 133, 136, 143, 145, 147-153) were analysed.

From 3674 abstracts 28 studies, including 7101 subjects, which met inclusion criteria were identified (Figure 3).

Figure 3: Flowchart describing selection of studies



2.3.1 Characteristics and quality assessment of included studies

17 studies (5319 subjects) described overall pain and 11 (1782 subjects) described specific pain subtypes. The majority of the studies which investigated a specific pain syndrome assessed headache (ten studies, 1581 subjects, one of which (147) included two patient samples). Study methodology and quality assessment are summarised in Table 5.

In each sample between 55%(7) and 96% (148) of subjects were female, between 30% (144) and 100% (147) had relapsing remitting MS, mean age was between 30.8 (147) and 54 (144) years, mean EDSS score was between 1.1 (147) and 5.3, (26) and mean disease duration was between 2.5 (25) and 23 (144) years . On quality assessment using our four pre-specified criteria, only eight studies described any control population (six contemporaneous, (26, 145, 148-151) two historical (11, 154)), four described blinding procedure of any sort, (150, 151, 153, 154) and five described follow-up. (7, 25, 133, 148, 150) Seventeen used at least one externally available validated instrument, of which nine (133, 147-149, 151-153) were headache studies referring to International Headache Society Criteria. (155, 156) Of overall pain studies, two studies (7, 146) met one criterion, four (11, 25, 26, 145) met two and none met more than two. Of pain subtype studies, four studies (134, 136, 147, 152) met one criterion, three (133, 149, 153) met two, three (148, 151, 154) met three and one (150) all four.

Table 5: Characteristics of Included Studies

			QUALITY ASSESSMENT				PAIN ASSESSMENT DETAILS			
authors	n= MS total	Overall proportion suffering pain	follow-up?	controls?	validated externally available assessment instrument?	blinding?	pain types studied	instrument(s) used	specific exclusions	allocated pain timeframe stratum ^a
GENERAL PAIN PREVALENCE STUDIES										
archibald et al [1]	85	0.53	NO	NO	NO	NO	general	structured interview	none recorded	Up to one month
beiske et al [2]	142	0.65	NO	NO	NO	NO	general	structured interview	primary headache	Up to one month
boneschi et al [3]	428	0.40	NO	NO	NO	NO	general	semistructured questionnaire	chronic pain lasting less than 6 months	Longer than one month
brochet et al [5]	68	0.74	YES	NO	YES	NO	bodily pain	SEP-59	none recorded	Up to one month
douglas et al [9]	219	0.67	NO	NO	NO	NO	general	piloted questionnaire booklet, interview	everyday pain - minor headaches, sprains and toothache	Up to one month
fryze et al [11]	104	0.70	NO	NO	NO	NO	general	authors' questionnaire	none recorded	Longer than one month
grasso et al [13]	128	0.48	NO	YES ^b	YES	NO	general	sfMPQ, VAS, component of SF36	VAS score less than 3	Up to one month
grau-lopez et al [14]	134	0.55	NO	NO	NO	NO	general	semi-structured interview	none recorded	Up to one month
indaco et al [18]	122	0.57	NO	NO	NO	NO	general	interview	chronic headache, pain syndromes relieved by analgesics	Longer than one month
kalia et al [20]	99	0.69	NO	YES ^c	YES	NO	any chronic	VAS, sfMPQ, component of SF36	chronic pain due to other diagnosis or trauma	Up to one month
kassirer, osterberg [21]	28	0.82	NO	NO	NO	NO	general	questionnaire	none recorded	Longer than one month
osterberg et al [32]	364	0.57	NO	NO	NO	NO	general, particularly central	postal questionnaire interview in person	back pain, tension headache, migraine, optic neuritis	Longer than one month
solaro et al [39]	1672	0.43	NO	NO	NO	NO	general	structured questionnaire	headache, acute pain due to ON, somatic pain other than back	Up to one month

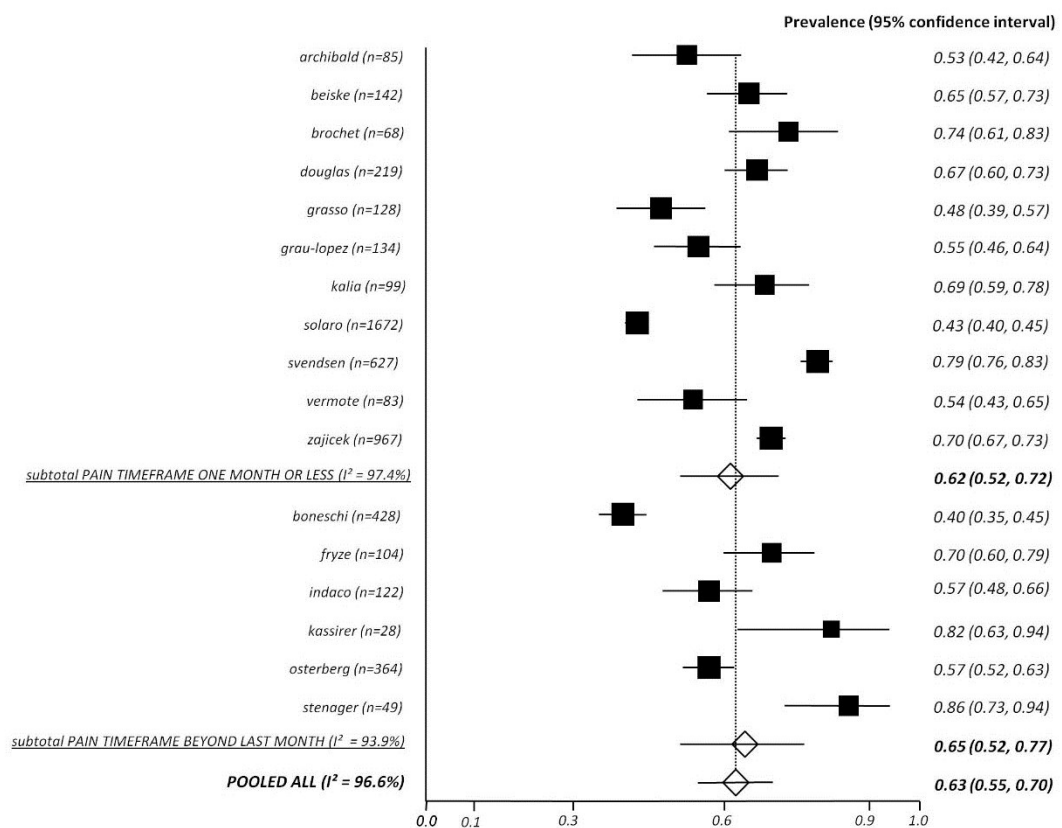
									pain, tendonitis, capsulitis	
stenager et al [41]	49	0.86	YES	NO	NO	NO	general	interview, examination	headache, minor pain relieved by analgesics	Longer than one month
svendsen et al [43]	627	0.79	NO	YES ^b	YES	NO	general	MPQ	none recorded	Up to one month
vermote et al [47]	83	0.54	NO	NO	NO	NO	general	questionnaire with elements of MPQ	headache, visceral pain	Up to one month
zajicek et al [51]	967	0.70	NO	NO	YES	NO	general	authors' questionnaire, SF36	none recorded	Up to one month
SPECIFIC PAIN SUBTYPE PREVALENCE STUDIES										
d'amico et al [7]	116	0.58	NO	NO	YES	NO	headache	authors' questionnaire, ^e	non-headache pain	Longer than one month
ergun et al (remission phase) [10]	34	0.74	NO	NO	YES	NO	headache	interview ^e	trigeminal neuralgia optic neuritis, other cranial neuralgia	Longer than one month
ergun et al (relapse phase) [10]	18	0.39	NO	NO	YES	NO	headache	interview ^e	non-headache pain	Up to one month
katsiari et al [22]	48	0.50	YES	YES ^b	YES	NO	headache	interview ^e	non-headache pain	Longer than one month
kister et al [23]	204	0.64	NO	YES ^c	YES	YES	migraine	multiple questionnaires	related to trauma, infection or medication	Longer than one month
pollmann et al [33]	82	0.65	YES	NO	YES	NO	headache	standardised questionnaire ^e	analgesic overuse headache	Longer than one month
putzki et al [36]	491	0.54	NO	YES ^b	YES	NO	headache	questionnaire ^e	non-headache pain	Longer than one month
rolak and brown [38]	104	0.52	YES	YES ^b	YES	YES	headache	authors' interviews, psychiatric interview ^f	optic neuritis, trigeminal neuralgia	Longer than one month
vacca et al [45]	238	0.51	NO	YES ^b	YES	YES	headache	semistructured interview ^e	non-headache pain	Longer than one month
villani et al [48]	102	0.62	NO	NO	YES	NO	headache especially primary headaches	authors' questionnaire ^e	non-headache pain	Longer than one month
villani et al [49]	144	0.64	NO	NO	YES	YES	headache especially primary headaches	authors' questionnaire ^e	probable migraine	Longer than one month
vazirinejad et al [46]	201	N/A ^d	NO	NO	YES	NO	related to potential rehabilitation needs	NEADL, MSQOL54, own questions	none recorded	Up to one month

a - period of interest over which pain occurrence was investigated, stratified into; up to and including one month prior to assessment, and longer periods prior to assessment (as described in text) b – contemporaneous controls c – historical controls d - overall pain prevalence not available (pain subtype prevalence data presented) e – based on International Headache Society Criteria (1988 or 2004 versions) f - based on definitions of Ad Hoc Committee on Classification of Headache (1962)	VAS – Visual Analogue Scale for pain MPQ – McGill Pain Questionnaire sfMPQ– short form McGill Pain Questionnaire NEADL – Nottingham Extended Activities of Daily Living scale SF36 – short form 36 scale MSQOL-54 – Multiple Sclerosis Quality of Life-54 scale SEP-59 – “Sclerose en Plaques-59” French Language scale derived from SF36 and MSQOL54
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2.3.2 Prevalence of pain overall

Pooled overall pain prevalence from 17 estimates (7, 11, 20-27, 44, 66, 142-146) was 62.8% (95%CI 55.1% to 70.3%). Pain prevalence stratified by study pain timeframe (for studies examining pain within the last month prior to assessment, and studies examining pain over longer periods) was 61.8% (95%CI 51.6% to 71.5%) and 64.7% (95%CI 51.7% to 76.7%) respectively (Figure 4).

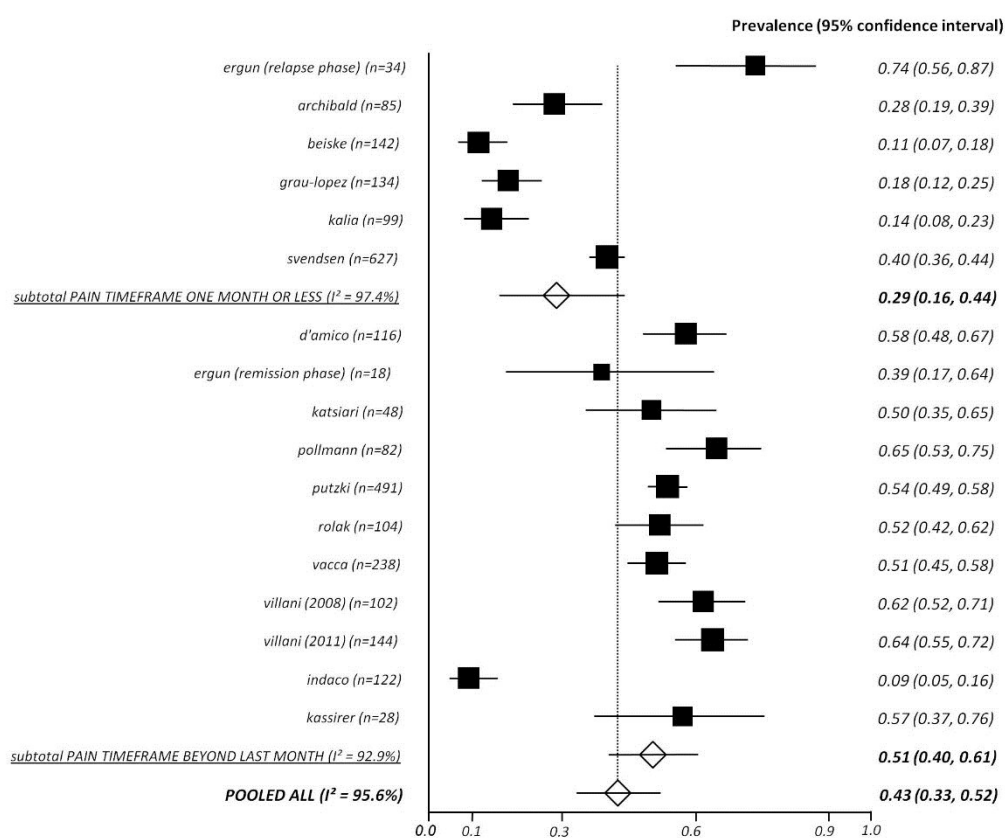
Figure 4: Overall prevalence of pain in multiple sclerosis (seventeen studies)



2.3.3 Prevalence of specific pain syndromes

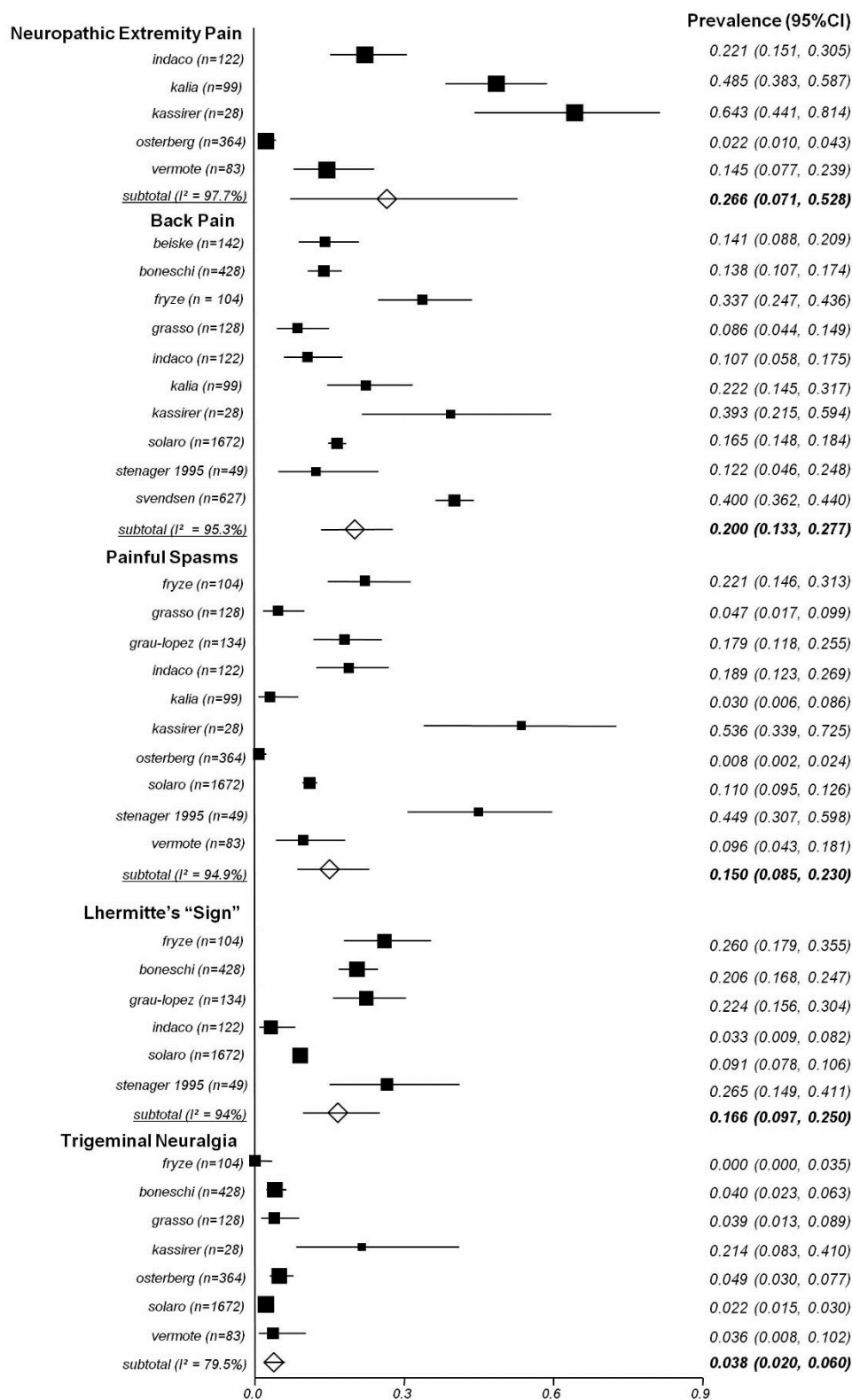
From 17 estimates of headache prevalence (11, 22, 24, 27, 44, 133, 136, 143, 145, 147-153) pooled prevalence was 42.5% (95%CI 33.2% to 52.1%). Headache prevalence stratified by study pain timeframe was 28.8% (95%CI 15.8% to 44.0%) for studies examining pain within the month prior to study, and 50.5% (95%CI 40.4% to 60.6%) for studies examining pain over longer periods (Figure 5).

Figure 5: Overall prevalence of headache, stratified by timeframe of assessment (seventeen estimates)



Pooled prevalences (95%CI) of specific pain syndromes were; neuropathic extremity pain 26.6% (7.1% to 52.8%), back pain 20.0% (13.3% to 27.7%), painful spasms 15.0% (8.5% to 23.0%), Lhermitte's sign 16.6% (9.7% to 25.0%) and trigeminal neuralgia 3.8% (2.0% to 6.0%) (Figure 6). There was insufficient data to allow pooled estimates for other pain syndromes (such as painful optic neuritis). Low risk of small study bias was found for all described estimates.

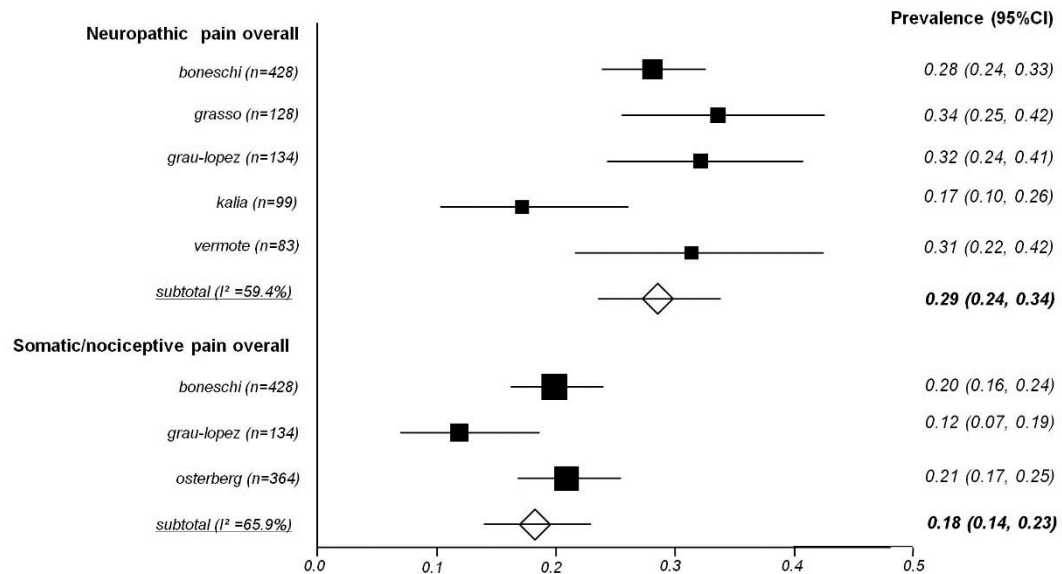
Figure 6: Prevalence of specific pain syndromes



Nociceptive and Neuropathic pain

Pooled overall prevalence of investigator-defined neuropathic pain was 28.5% (23.5% to 33.8%), and of somatic/nociceptive pain 18.2% (14.0% to 23.0%) (Figure 7).

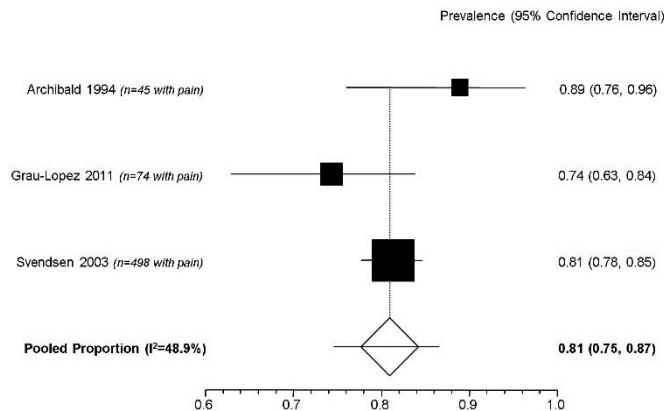
Figure 7: Reported aetiology of pain syndromes



Pain at more than one site

Three studies which explicitly described prevalence of pain at multiple bodily sites were found (22, 44, 145). Random effects meta-analysis suggested that, in these three studies, 81% of those reporting pain (95% confidence interval 75 to 87%) reported pain at multiple sites (Figure 8).

Figure 8: Prevalence of pain occurring at multiple body sites



Comparison of pain prevalence in MS subtypes

In an additional post-hoc analysis pain prevalence was further analysed in the few studies detailing the number of subjects with relapsing remitting MS (RRMS), primary progressive MS (PPMS) and secondary progressive MS (SPMS) (141). This data was available in nine studies of overall pain (7, 11, 20-24, 144, 146) and four headache studies (149, 151-153). Of these studies five studies of overall pain (20-23, 146) presented pain prevalence separately for each disease subgroup. For these five studies pooled pain prevalence in relapsing-remitting disease was 50.0% (95%CI 35.4% to 64.5%) (five studies (20-23, 146) , 2089 subjects with RRMS, I² 97.1%). In secondary progressive MS pooled pain prevalence was 69.8% (95%CI 54.7% to 83.0%) (five studies (20-23, 146) , 673 patients with SPMS, I² 92.6%) and for primary progressive MS pooled pain prevalence was 70.3% (95%CI 59.9% to 79.8%) (five studies (20-23, 146) , 393 patients with PPMS, I² 72.4%).

Of the four headache studies detailing the number of subjects with relapsing remitting, primary progressive and secondary progressive MS (149, 151-153) only two presented headache prevalence separately for each subgroup (152, 153). Given the low number of studies weighted meta-analysis was not carried out, however in each study separately headache prevalence in RRMS was 74.7% (83 subjects with

RRMS, 95%CI 64.0% to 83.6%) (152) and 76.3% (118 subjects with RRMS, 95%CI 67.6% to 83.6%) (153); and in SPMS 63.2% (19 subjects with SPMS, 95%CI 38.4% to 83.7%) (152) and 65.4% (26 subjects with SPMS, 95%CI 44.3% to 82.8%) (153). No subjects in these two studies were classified as having PPMS. Although the limited number of studies and subjects included in this post-hoc analysis did not suggest a statistically significant difference in overall pain prevalence or headache prevalence according to disease subgroup, given the small number of studies reporting pain prevalence by MS subgroup, a clinically important difference between groups cannot be excluded.

2.3.4 Associations of study-level pain prevalence: meta-regression analysis

No studies of overall pain which used investigator blinding of any type were identified, and only one study of headache prevalence describing an inpatient population. The amount of estimate heterogeneity accounted for by these variables could not therefore be by meta-regression.

For overall pain estimates none of the pre-specified methodological or sample demographic variables significantly explained estimate heterogeneity. For headache estimates only the study pain timeframe (up to a month prior to assessment, compared to greater than a month) accounted for a significant proportion of between-study heterogeneity. Timeframe of assessment of longer than one month prior to assessment was associated with higher headache prevalence than estimates assessing only headache in the preceding month (Table 6).

Table 6: Meta-regression analysis of study- and population- level associations with pain prevalence estimates

Studies analysing overall pain prevalence (total 17 estimates)				Studies analysing headache prevalence (total 17 estimates)		
	Number of studies where data available	Adjusted R- squared ^c	P value	Number of studies where data available	Adjusted R- squared ^c	P value
Study variables						
Blinding	No blinded study identified, therefore meta-regression not carried out			17 <i>3 blinding used, 14 blinding not used</i>	0.73%	0.328 ^a
Outpatient population studied	17 <i>13 outpatient sample, 4 inpatient sample</i>	-3.12%	0.497 ^a	Only one inpatient study identified, therefore meta-regression not carried out		
Pain timeframe (pain within one month/longer than one month from time of assessment)	17 <i>10 pain within last month only, 7 longer timeframe</i>	-4.81%	0.506 ^a	17 <i>6 pain within last month only, 11 longer timeframe</i>	32.13%	0.012 ^a
Demographic variables						
EDSS (mean)	7	-16.22%	0.675 ^b	10	43.86%	0.026 ^b
Proportion female gender in population	16	-7.05%	0.868 ^b	17	-5.02%	0.667 ^b
Proportion progressive MS in population	13	-6.08%	0.617 ^b	11	15.05%	0.145 ^b
Disease duration (mean)	12	-10.30%	0.768 ^b	12	28.12%	0.051 ^b

^a threshold significance value $p < 0.017$ for individual analyses, based on Bonferroni correction (3 comparisons) with $p < 0.05$ significance threshold

^b threshold significance value $p < 0.012$ for individual analyses, based on Bonferroni correction (4 comparisons) with $p < 0.05$ significance threshold.

^c negative adjusted R^2 values may arise in the case of small sample sizes where R-squared value is less than expected by chance

In an additional post-hoc meta-regression analysis, separately examining the proportion of patients with SPMS and PPMS as independent variables did not significantly explain the observed heterogeneity in overall pain estimates (nine studies; SPMS: adjusted R^2 -10.19%, p value 0.586; PPMS adjusted R^2 17.91%, p value 0.172; threshold p value for both comparisons 0.0083 following Bonferroni correction for a total of six demographic variables). Post-hoc meta-regression analysis was not carried out using these independent variables for headache studies, as insufficient study numbers were available.

2.3.5 Pain incidence

No estimates of pain incidence (defined as rates of first occurrence of any pain syndrome) were found.

2.3.6 Pain prevalence at MS disease course milestones

Pain prevalence prior to disease onset

No prospective studies describing prevalence of overall pain prior to MS onset were identified (acknowledging the methodological challenges of any such study).

Pain prevalence at disease onset

One study prospectively estimated pain prevalence soon after diagnosis (73.5% prevalence of any pain).(25) Mean disease duration at assessment was 30.5 months (range 3 to 202 months).

Pain prevalence at relapse

Only one study prospectively analysed pain at relapse, reporting headache prevalence of 38.9% (number of subjects with headache =7, 5 primary stabbing headache, 2 migraine). (147)

Pain prevalence during disease evolution

Only two studies prospectively examined overall pain evolution with disease progression. (7, 25) Both describe a population of mixed MS disease types. Brochet and colleagues (25) studied 68 subjects with early MS over 2 years. 33% of RRMS and 45% of PPMS subjects reported clinically significant pain at all time points. Pain prevalence appeared to decrease over time, however this trend was not statistically significant. Stenager and colleagues (7) studied 70 subjects at baseline, and reassessed 49 of these after 5 years. They found a significant increase in prevalence of overall pain and of several pain syndromes, particularly in subjects with deteriorating EDSS. Brochet and colleagues report no loss to follow-up, whereas Stenager and colleagues report loss to follow-up of 30%.

2.3.7 Further literature since original searches, identified by cited reference search

Estimates of pain prevalence

2.3.7.1.1 Drulovic and colleagues, 2015

Drulovic and team's useful multicentre cross-sectional study carried out in Serbia, Bosnia & Herzegovina, and Croatia post-dated the systematic review described above (41). This prospective study of 650 people with MS (revised McDonald criteria (76)) administered a semistructured questionnaire as well as other instruments (including HADS (157) and EDSS(158)). Lifetime prevalence of pain was found to be 66.5%, and point prevalence 44.3%. Pain prevalence estimates were compatible with the findings described above. Neuropathic extremity pain was found in 53% of subjects (my estimates 26.6%, with 95% CI 7.1% to 52.8%). The prevalence of pain syndromes was similar.

Age, progressive disease subtype, disability, depression and anxiety were all significantly associated with pain. In a multivariate analysis, anxiety was the main independent predictor of the presence of pain.

2.3.7.1.2 Khan and colleagues, 2007

Khan and colleagues (159) carried out a single-centre cross-sectional study of pain prevalence in 94 people with definite MS based in a neurological clinic in Australia. This study was published in 2007 and was excluded from the systematic review described above because of the description of inclusion criteria described in the study. On review, however, the inclusion criteria for the systematic review were probably met.

Specifically the authors describe that “participants were recruited from the MS database... participants included on the database were recruited through the MS society and ...neurologists”. It was concluded that not all participants therefore had MS diagnosed by a neurologist. The authors however separately state “the source of these participants (ie participants in their current study) was a pool of persons not solely based on membership in the MS society or hospital clinics. These persons... (had a) confirmed diagnosis of MS based on Poser’s criteria...”(159). The content of this study is therefore discussed as it is likely that Poser’s criteria would have been applied by a neurologist.

This study found that 65% of people with MS had chronic pain, most frequently in the lower limbs. Most pain was dysaesthetic in nature, mild to moderate in severity, and at more than one location. Those with higher pain severity had more disability, and more frequent health care visits. There was a significant difference reported between people with and without pain, and people with less and more severe pain, in a variety of outcomes including quality of life and independent living. Those with and without pain did not differ significantly in terms of gender balance, age, disease duration, severity or stage of MS.

Pain in MS relapse

Pain in MS relapse has been very rarely specifically addressed. My systematic review identified no prospective studies in people with definite MS.

2.3.7.1.3 *Silva and colleagues, 2015*

One recent cross-sectional clinic based study , by Silva and colleagues (160) administered a questionnaire to 100 MS patients diagnosed by McDonald criteria. The study was sponsored by a pharmaceutical company. The patients attended the Antonio Pedro University Hospital (Brazil). Assessment included the DN4 (161, 162) and LANSS instruments, as well as measures of anxiety and depression (Beck Depression Inventory and Anxiety Inventory respectively (163)). The authors report that increased relapses in their sample were associated with a substantial reduction in the prevalence of pain in the sample. The presence of pain was independent of anxiety, depression and EDSS. A large number of analyses were carried out and the effects described apply to patient subgroups restricted by number of relapses. Interpretation of these findings remains unclear, and available information relating to pain in MS relapse remains very limited.

Longitudinal characterisation and incidence of pain in MS

Information on longitudinal characterisation of pain in MS, as described above, is limited. Some papers published since the time of the above review offer further perspectives.

2.3.7.1.4 *Khan and colleagues, 2013*

Khan and colleagues extended a previous cross-sectional study in 2007 (described above (159)) to include a further assessment of the same patient group after a 7-year interval. 74 of the 94 people described above were reviewed (21% loss to follow-up). At the second time point, 13.8% more participants reported chronic pain than at the baseline assessment. No significant differences were reported in average pain intensity, though more subjects reported high pain intensity. Greater disability was reported at the second time point, and at the second time point the subjects reported more healthcare utilization, and use of more non-pharmacological pain therapies.

2.3.7.1.5 *Fiest and colleagues, 2015*

Fiest and colleagues, writing on behalf of the “Canadian Institutes of Health Research team on the Epidemiology and Impact of Comorbidity on Multiple Sclerosis” (ECoMS) (37) recruited 949 people with definite MS from four Canadian centres, who subsequently underwent review at 1 year and 2 years after baseline

visit. Loss to follow-up was low at 5.4%. This study provides the first annualised incidence rate of pain in MS. Over 2 years, the incidence of disruptive pain (defined using the Health Utilities Index (164)) was 31.3%.

The researchers also examined the impact of comorbidity, with particular reference to pain which disrupted daily living, and found that fibromyalgia, rheumatoid arthritis, irritable bowel syndrome, migraine, chronic lung disease, depression, anxiety, hypertension and hypercholesterolaemia were all associated with the presence of disruptive pain.

The mechanism(s) whereby these comorbidities could contribute to, or modulate, pain interference, is not always apparent, though some of these disorders are in themselves often painful. Specifically, anxiety was associated with worsening pain over time. Depression was associated with a 1.58-fold increased prevalence of pain at baseline. This detailed and thorough paper offers useful new information regarding pain incidence and comorbidity in particular. The authors did not however examine the characteristics of pain in their sample, and, in common with other studies described, used a clinic-based population, which may limit generalizability of their conclusions.

2.3.7.1.6 Kister and colleagues, 2013

Kister and colleagues, writing in 2013 (165) report patient symptom burden in a very large population of around 26 thousand North American patients who self-reported a diagnosis of MS, and were included in the NARCOMS database. Follow-up was available over 30 years.

The figures presented suggest that at onset, around 59% of subjects reported pain. After 15 years, around 77% reported pain, and after 30 years, around 85%. There was, however, little change in the distribution of pain severity over time (similar to the data presented in the same article for depression, sensation, vision and cognition). Assessment of pain used a brief questionnaire asking subjects to grade presence of severity and pain on a numerical rating scale from 0 to 5 (normal, minimal, mild, moderate, severe, and total disabling pain). Each severity grade was further defined in terms of limitation in everyday activities.

Although those included in this study may not all have MS, as inclusion in the database was by self-report, and the phenotyping of pain is not detailed, the large sample size and combination with other data provide convincing estimates of the prevalence of pain in North America, and increasing prevalence over the MS disease course

2.4 Discussion

The prevalence of pain in MS has been found to be around 63%. It is composed of a variety of pain syndromes and mechanisms. There is significant heterogeneity associated with prevalence estimates, though examined aspects of study design and sample populations did not significantly explain heterogeneity in overall pain estimates. It is most likely that variable study design and execution (even within this selected study group) contributes to this heterogeneity. Study findings also included that characterisation of pain during the MS disease course is limited, and that incidence has not been studied. Therefore, while pain is common in MS, its relationships to disease course are poorly quantified.

Study findings and implications, and study methodology, are discussed in detail in the Discussion (Chapter 7).

Further studies published since this systematic review have found similar pain prevalence estimates, and the natural history of pain in MS at time of writing is perhaps better characterised than previously (37, 165)

Chapter 3 Neuroimaging of Pain Syndromes in Adults with Multiple Sclerosis: Systematic Review

3.1 Introduction

Neuroimaging plays a key role in the diagnosis of multiple sclerosis, and in current understanding of pathophysiological mechanisms, and their links to clinical manifestations of MS.

This work aimed to define and to assess the existing literature describing associations of pain syndromes in people with MS, with neuroimaging findings.

Please see the thesis Introduction (page 1) for a detailed discussion of the background to this work, aims and hypotheses.

3.1.1 Peer-reviewed publication related to this work

Sections of this work were published as

D Seixas*, P Foley*, J Palace, D Lima, I Ramos, I Tracey

- “Pain in multiple sclerosis: A systematic review of neuroimaging studies”
- NeuroImage: Clinical Volume 5, 2014, p322-331 (166)
- (* = joint first authors)

3.1.2 Contributions

Peter Foley (PF) and Daniela Seixas (DS) independently conceived of the study concept for this systematic review. DS carried out the first literature searches and wrote the first draft of the peer-reviewed journal article (referred to above). PF had substantial input at all stages of execution of this study, and has written this thesis chapter which includes revised assessment of the identified studies.

3.2 Materials and Methods

The primary outcome of interest was the radiological evidence for the aetiology of any pain syndrome in MS.

3.2.1 Literature search and selection criteria

Search strategies

PubMed and Scopus were searched from their inception dates (1977 and 1960, respectively), to the 2nd April 2013. Medical Subject Heading (MeSH) keywords were used for the PubMed search, and were combined with Boolean operators. Initial searches were carried out by DS, and subsequently updated by PF.

Firstly, a search for studies concerned with pain and multiple sclerosis was performed. Keywords were the MeSH terms “pain” AND “multiple sclerosis” (see Table 7).

A second search for studies using any neuroimaging techniques of interest was performed. The keywords “magnetic resonance imaging”, “positron-emission tomography” and “tomography, emission-computed, single-photon” were combined using the OR operator.

The first and second searches were then combined using the AND operator to search for articles concerned with both pain associated with MS, and relevant neuroimaging techniques. All MeSH terms were “exploded” to include all subheadings. Please see Table 7 below for a summary:

Table 7: PubMed search strategy

Search topic	Search terms
	(
Pain	(pain[MeSH Terms])
	AND
Multiple Sclerosis	(multiple sclerosis[MeSH Terms])
)
	AND
	(
MRI	(magnetic resonance imaging[MeSH Terms])
	OR
PET	(positron emission tomography[MeSH Terms])
	OR
SPECT	(tomography, emission computed, single photon[MeSH Terms])
)

Search terms for the Scopus search were combined in the same manner as described above. Search terms were generated by individually listing MeSH subheadings generated by the “index list” function in PubMed for each keyword, as well as the MeSH keyword itself. Please see Appendix: Scopus Search Terms for further detail.

Reference lists in identified manuscripts were also hand-searched, and specialists in pain neurobiology (University of Porto, University of Oxford) were consulted in order to identify additional relevant material.

3.2.1.1.1 Update of literature search for thesis chapter

For preparation of this thesis chapter, the PubMed and SCOPUS searches were rerun by PF, in May 2016. New papers not included in the original systematic review were identified.

Inclusion and Exclusion Criteria

3.2.1.1.2 *Definition of multiple sclerosis*

Papers stating a definite diagnosis of Multiple Sclerosis in adult humans were included. The use of specific diagnostic criteria was not mandatory (76, 131, 132). Use of diagnostic criteria was however recorded and explored post-hoc (see below).

Studies of childhood onset MS were excluded (because of possible clinical and epidemiological differences from MS of adult onset (135)) and studies of other demyelinating disorders (such as neuromyelitis optica, or clinically isolated syndrome of CNS demyelination).

3.2.1.1.3 *Definition of pain syndromes*

Any pain syndrome described in the identified study as associated with MS was included. Searches were not limited to specific pain syndromes, in order to limit possible biases towards any specific pain syndromes.

3.2.1.1.4 *Language*

Only English language studies were included. Studies published in languages other than English were not included, because of lack of ready availability of translators for studies published in other languages, and because of an anticipated low number of studies published in other languages.

3.2.1.1.5 *Imaging modalities*

Studies using MRI, PET or SPECT imaging in human adults, as described above, were included. Studies using CT (computed tomography) were excluded because of a lack of ability to distinguish relevant CNS neuroimaging findings (77), in particular demyelinating MS lesions.

3.2.1.1.6 *Other exclusion criteria*

Re-published data, and review articles were excluded.

Process of review by researchers

At first execution of the literature searches, DS reviewed the titles and abstracts of identified studies, and excluded duplicate references.

Two reviewers (DS, PF) independently reviewed potentially relevant articles following the initial literature search. Disagreements were resolved by consensus.

For purposes of producing this thesis chapter, reasons for excluding potentially relevant articles were extracted from the available data. Reasons for excluding titles and abstracts at first review were not available for all studies at the screening stage, and were estimated from available data.

3.2.2 Data Extraction and Analysis

Data Extraction

For each included study methodological data was extracted including: pain syndrome(s) studied, basis for diagnosis of pain syndrome (where stated), basis for diagnosis of multiple sclerosis (where stated), description of imaging methods (specifically including image acquisition, and image analysis), and methods used to investigate temporal associations between any radiological findings and occurrence of a pain syndrome. The latter included use of serial imaging and contrast imaging.

Factors relevant to quality assessment of each study were also recorded:

Quality assessment of included studies

Methodological quality of experimental studies was assessed using 12 criteria relevant to our review objectives (adapted from Campbell and colleagues (167, 168)). These criteria were chosen because the design of our studies was not identical, necessitating a flexible approach to methodological appraisal (169). Given the low number of identified studies, studies were not excluded on the basis of quality assessment results.

These criteria were as follows:

1. Clearly stated research objective,
2. Clear description of recruitment procedure,
3. Clear description of inclusion/exclusion criteria
4. Description of participation rates
5. Clear description of sample demographics
6. Clear description of imaging protocol

7. Clear description of pain measurement instruments
8. Participation rate above 70% at baseline
9. Image interpretation carried out without knowledge of subjects' pain status (ie blinded to subject pain status)
10. Use of multivariate analysis (where appropriate)
11. Reporting of strength of effect
12. Acknowledgement of study limitations

While a “score” for each study has been calculated, by simply summing the number of quality assessment items identified in each study from a potential total of 12, this should not be interpreted as an ordinal scale, but rather as a framework for discussion where relevant (see below).

Categorisation of studies

Studies were identified as case reports, case series, or experimental studies (defined here as any study with hypothesis-driven experimental design). These descriptions were used to facilitate discussion and assessment of the included studies.

In discussion, priority is given to experimental studies.

Statistical analysis

Descriptive statistical analysis was carried out where appropriate, using Microsoft Excel, or R version 3.1.2 implemented in R Studio, in a Windows 7 environment.

3.2.2.1.1 *Synthesis of results*

Meta-analysis was not carried out as study methodologies were not sufficiently similar, and/or appropriate numerical outcomes were not available.

3.2.2.1.2 *Ethics committee approval*

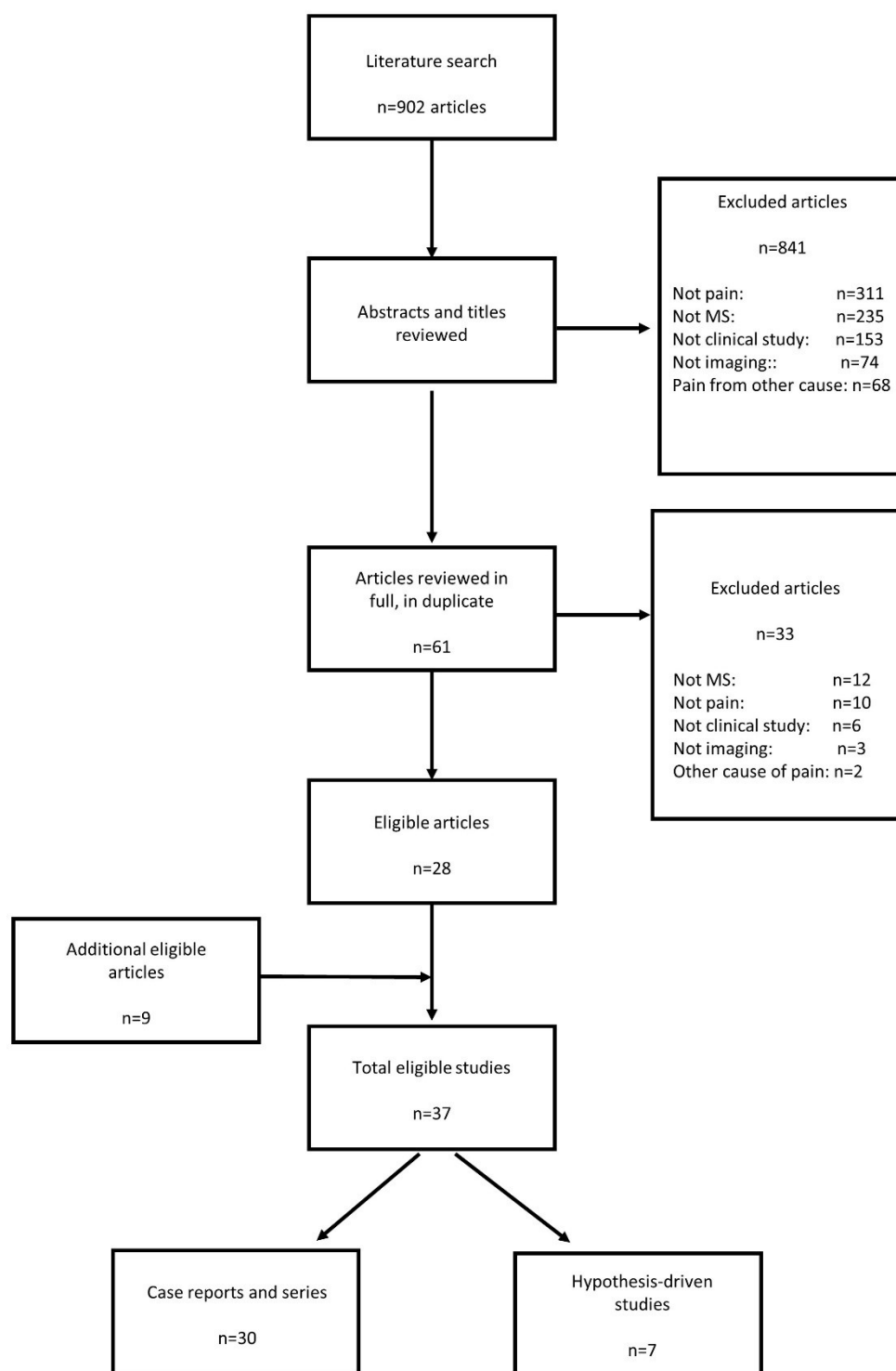
This work was not submitted to an ethics committee because it is a systematic review of the literature. We followed PRISMA guidelines for systematic reviews (170).

3.3 Results

From 902 abstracts 37 studies which met inclusion criteria were identified (117, 154, 171-206) Of these, 16 were case reports (172, 173, 175, 177, 180-182, 186, 189, 193, 194, 196, 199-201, 207), 14 were case series (171, 178, 179, 184, 185, 187, 192, 195, 198, 202, 204-206, 208) and seven were experimental studies (154, 174, 176, 183, 188, 191, 197).

Please see Figure 9 below:

Figure 9: Flowchart describing selection of studies



3.3.1 Design of experimental studies

Design of these seven experimental studies is detailed in Table 8 (below).

Four studies compared people with MS with and without pain (154, 176, 183, 191), One used a control group of people with pain but without MS (197), one used a control group of healthy volunteers (188) and one used a combination of MS and non-MS control groups (174). All examined one specified pain syndrome.

Table 8: Summary of experimental studies identified by this review

First author	Study design	Study population, description and size	Main study focus	Pain assessment techniques	Imaging techniques	Main findings
Balasa et al (197)	Retrospective, case-control	20 patients with TN (10 with MS and 10 without MS)	Evaluation of clinical differences in TN presentation and pharmacological treatment response. Contrast between patients with and without MS	International Headache Society Classification (156) Barrow Neurological Institute score of clinical pain intensity (209)	MRI (1 T), no image acquisition or reading protocols defined	MS patients had earlier onset TN, probably secondary to lesions in the trigeminal pathways. Overlapping clinical characteristics and treatment response when compared to non-MS TN
Crucchi et al (191)	Prospective, case-control	130 MS patients (50 with TN, 30 with sensory trigeminal disturbances, 50 controls without trigeminal disturbance)	Causes and mechanisms of MS-related TN	International Headache Society Classification (156) Neurological examination including sensory and trigeminal reflex testing	MRI, dedicated image acquisition protocol although not fully described. Voxel-based brainstem analysis, read by neuroradiologists	The onset ages of MS and trigeminal symptoms were older in the TN group, and most patients in TN and non-TN groups had abnormal trigeminal reflexes. In TN group, the highest probability of brainstem lesion was in the pontine trigeminal primary afferents
Deppe et al (188)	Prospective, case-control	1 MS patient, 100 healthy controls	Thalamic DTI measurements in person with MS developing neuropathic	Describes the pain syndrome as "episode of central pain and abnormal	MRI (3 T, well described imaging protocol and post-processing). Data was	Temporary increase of the fractional anisotropy in the thalamus contralateral to pain

First author	Study design	Study population, description and size	Main study focus	Pain assessment techniques	Imaging techniques	Main findings
			pain syndrome: comparison with controls.	somatosensory and thermal sensations on the right hand side of the body”	obtained from a pilot for a clinical trial. ROI analysis of the thalami.	symptoms; a causative role is suggested
Gee et al (183)	Retrospective identification of imaging from clinical database. Combined with prospective patient questionnaire	277 MS patients including 154 with headache	To determine if the prevalence of migraine-like headache in MS patients was associated with plaques in brainstem or other locations	International Headache Society Classification (155), tailored questionnaire	MRI (1.5T). predefined reading protocol	The presence of a midbrain plaque was associated with an increased likelihood of headache with migraine characteristics ; lesions in other locations and lesion load were not associated with headache prevalence
Kister et al (154)	Prospective, cross-sectional	204 MS patients, with and without migraine.	To assess the relative frequency of migraine in MS, and to compare clinical and radiographic characteristics in MS patients with and without migraine	International Headache Society Classification (156), in-house headache questionnaire, migraine severity assessed with Migraine Disability Assessment tool (210)	MRI (0.6, 1.5 and 3 T), image acquisition protocol defined (T2-w and pre and post-contrast T1-w), images read by a neurologist and an expert in MS neuroradiology	Migraine frequency was higher in MS patients than in controls, and was more symptomatic. No difference in number or distribution of plaques, or enhancing lesions between migraine and no-migraine groups
Svendson et al (176)	Prospective case-control	23 MS patients (13 with	To study location of plaques in	Structured pain interview,	MRI (1.5 T), image acquisition	No association between

First author	Study design	Study population, description and size	Main study focus	Pain assessment techniques	Imaging techniques	Main findings
		neuropathic pain, 10 with painless sensory disturbance)	MS patients with sensory disturbances with and without pain, and to ascertain if deafferentiation of spinothalamic tract was more common in the patients with pain	pain location on body diagram, neurological examination	protocol defined (brain – sagittal T1-w and T2-w and axial FLAIR, spine – sagittal T1-w and STIR axial T2-w), read by a neuroradiologist according to defined protocol	central pain and site of demyelination was found; central pain was associated with allodynia, suggesting central hyperexcitability
Tortorella et al (174)	Retrospective case-control	58 patients with migraine and without MS and 79 MS patients (37 with, and 42 without migraine)	Evaluate if red nucleus, substantia nigra and periaqueductal grey matter were involved by MRI-detectable structural abnormalities in migraine patients, and to investigate their frequency and extent in MS patients with migraine	International Headache Society Classification (156)	MRI (1.5 T), defined image acquisition protocol (axial PD/T2-w), read by two observers using a defined reading protocol	Brainstem lesions were frequent in non-MS migraine, but did not seem associated with aura. Demyelinating lesions in the red nucleus, substantia nigra and periaqueductal grey matter might be among the factors responsible for migraine in MS
Abbreviations 1T, 1.5T, 3T = One, one point five or three tesla MRI acquisitions T1-w and T2-w = T1 weighted and T2 weighted MRI acquisitions PD = proton density MS = multiple sclerosis MVD = microvascular decompression TN = trigeminal neuralgia TOF = time of flight						

3.3.2 Quality Assessment of included studies

On quality assessment, the number of quality criteria identified in each study was not normally distributed, and was positively skewed. The median number of criteria fulfilled by included experimental studies (n=7) was eight (range 3-12). One study (154) fulfilled all criteria (Table 9).

Table 9: Quality assessments of experimental studies

First Author	Research objective	Recruitment procedure	Incl/Excl criteria	Demo-graphics	Parti'n rates	Pain measures	Imaging protocol	Blinded analysis	Strengt h of effect	Multivariat e analysis	Limitations discussed	P'ticipation over 70%
Balasa (197)	YES	YES	NO	NO	NO	YES	NO	NO	NO	NO	NO	NO
Cruccu (191)	NO	YES	YES	YES	YES	YES	NO	YES	NO	NO	YES	NO
Deppe (188)	YES	YES	YES	YES	NO	NO	YES	NO	NO	NO	YES	NO
Gee (183)	YES	YES	YES	YES	YES	YES	NO	YES	YES	YES	YES	NO
Kister (154)	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES
Svendse n (176)	YES	YES	YES	YES	YES	YES	YES	YES	NO	NO	NO	NO
T'torella (174)	YES	YES	YES	YES	NO	YES	YES	YES	NO	NO	YES	NO

All identified studies used conventional structural MRI apart from one, which investigated a lateralised pain syndrome in a single patient using serial diffusion tensor imaging (DTI) (188). No studies used functional imaging (functional MRI, SPECT or PET).

Field Strength

There were significant limitations in the description of imaging methodology in many studies. Overall, field strength was specified in 15 studies (39% of all studies) (172, 173, 175, 179, 180, 182, 184, 187-189, 191-194, 196, 198-202, 204, 205, 207) . Of these 15 studies, one Tesla (1 T) scanners were used in two studies (190, 197), 1.5 T scanners were used in nine studies (171, 174, 176-178, 183, 185, 206, 208) and 3 T scanners were used in two of the most recent studies (188, 199) (Table 10, see below) . Scanners of varying field strengths were employed in two studies (0.6 T, 1.5 T and 3 T (154), and 0.5 T and 1.5 T (195)).

MRI protocols

Of all the included studies, MRI protocols were described in 15 (39%) (172, 173, 175, 177-184, 186-189, 191-194, 196, 197, 199, 200, 202, 204, 205, 207). Of these 15, five described the scanner, 11 mentioned the field strength, 14 described the type of sequences (although only nine of these described all sequences), and nine described the sequence parameters used (although only four of these described all sequence parameters of all sequences). Imaging methodology was relatively better described in the seven experimental studies (please see Table 10).

Table 10: Description of MRI imaging techniques in included studies

MRI image acquisition	Papers
Scanner	Deppe 2013; Meaney 1995; Svendsen 2011; Gass 1997; Eldridge 2003
Field strength	Andrade 2012; Deppe 2013; Meaney 1995; Svendsen 2011; Gass 1997; Eldridge 2003; Kister 2010; Tortorella 2006; Broggi 2004; da Silva 2005; Yetimalar 2008
Sequences All the sequences used	Deppe 2013; Haas 1993; Meaney 1995; Kister 2010; Tortorella 2006; Svendsen 2001; Broggi 2004; da Silva 2005; Gass 1997
Some of the sequences used	Andrade 2012; Athanasiou 2005; Donat 2011; Eldridge 2003; González-Quintanilla 2012
Sequence parameters All sequences and parameters described	Tortorella 2006; Svendsen 2011; da Silva 2005; Gass 1997
Some sequences used and/or some parameters	Deppe 2013; Haas 1993; Meaney 1995; Broggi 2004; Athanasiou 2005

3.3.3 Diagnosis of multiple sclerosis in included studies

Criteria used to confirm the diagnosis of MS were explicitly stated in 17 of the 34 studies (these included 2010 revisions to the McDonald (76) criteria (181, 188); revised McDonald (131) criteria (154, 182, 184, 191, 199); McDonald (130) criteria (171, 193, 197, 202); Poser (132) criteria – (195, 202, 206, 207); and Rose (211) criteria (205).

Subtype of MS

The subtype of MS in subjects was not fully described in 14 studies (154, 171, 179, 181, 184-186, 191, 194, 197, 198, 204-206). It was relapsing-remitting in 16 (173, 175, 177, 178, 180, 182, 187, 188, 193, 196, 199-202, 204, 207). Six studies included patients with various MS subtypes (174, 176, 183, 190, 195, 208).

3.3.4 Pain syndromes assessed in included studies

All studies examined either neuropathic pain or headache. No studies investigating nociceptive/somatic pain, or psychogenic pain (2, 5) were found. Most studies (n=28, 74% of total) focused on headache or facial pain syndromes, and the remainder on bodily pain (eight studies, 21% of total), except for two studies (6%), which included both patients with headache/facial pain and body pain (171, 176) .

Please see Table 11 (below).

Table 11: Pain syndromes assessed in included studies

Type of pain syndrome	Study
Headache Migraine	Fragoso 2007; Kister 2010; Tortorella 2006
Cluster headache and other trigeminal autonomic cephalalgias	Cluster headache – Gentile 2007 Cluster-like headache – Donat 2011; Leandri 1999 Cluster-tic syndrome – González-Quintanilla 2012 SUNCT – Davey 2004; Vilisaar 2006 Probable trigeminal autonomic cephalalgia with allodynia – Liu 2008
Cranial neuralgias and central causes of facial pain	Glossopharyngeal neuralgia – Carrieri 2009; Minagar 2000 Occipital neuralgia – de Santi 2009 (3 cases) Painful third nerve palsy – Bentley 2002 Trigeminal neuralgia – Meaney 1995; Nakashima 2001; Cordella 2009; Athanasiou 2005; Pichiecchio 2007; Balasa 2010; Cruccu 2009; Broggi 2004; da Silva 2005; Gass 1997; Eldridge 2003
Other headache, cranial neuralgia, central or primary facial pain	Atypical trigeminal neuralgia/facial pain – Tanei 2010 Headache – Alstadhaug 2007; Haas 1993
Body pain Pseudo-radicular pain	Cervical – Tosi 1998 Sciatica – Marchettini 2006 Various levels – Ramirez-Lassepas 1992
Dysaesthetic pain	Burkey 2010; Deppe 2013; Hellwig 2006, Svendsen 2011
Pain and painful itching	Hellwig 2006
Painful tonic spasms	Andrade 2012
Visceral pain	Marchettini 2006
Various	Yetimalar 2008

3.3.5 Imaging correlates of pain syndromes in included studies

Measures of volume (brain or spinal cord)

No study used volumetric techniques to estimate volume or atrophy of CNS structures.

Lesion location

All studies described MS lesions thought to be responsible for pain syndromes, or searched for MS lesions in neuroanatomical areas felt to be linked to specific pain syndromes.

Table 12 describes lesion locations in the 25 included case reports and series. Of these, 21 describe demyelinating lesions in areas thought likely to be responsible for a pain syndrome. Four (189, 193, 198, 204) did not find demyelinating lesions thought likely to be responsible.

Lesions were identified in the CNS (compatible with central neuropathic pain) in 21 studies. Of these, lesions were located in the spinal cord in six studies (173, 179, 187, 194, 200, 202) (three cases in de Santi and colleagues' study (187), two cases documented with MRI in Hellwig and colleagues' study (202) and five in Marchettini and colleagues' study (179)). Lesions were identified in the brainstem in 13 studies (172, 175, 178, 180-182, 184, 186, 192, 196, 201, 206, 207), in the thalamus in one study (188) and in multiple locations throughout the pyramidal tract in another study (199).

All identified brainstem lesions corresponded to headache disorders, except for a lesion in the cerebral peduncle (among other lesions identified in the pyramidal tract) in a case of painful tonic spasms of the upper limb (199). The reported spinal cord lesions corresponded either to headache disorders (two studies reporting high cervical lesions at C1 or C2 levels (187, 200)) or to body pain (four studies) (Table 12). Lesions including both the peripheral and central nervous system were described in one study (which described lesions involving the brainstem and trigeminal nerve (177)).

Four studies found incidental structural lesions, which were unrelated to MS but felt to explain neuropathic pain syndromes (179, 185, 198, 206). These included neurovascular contacts in the case of trigeminal neuralgia (185, 198, 206), and lumbar spine degenerative disease (179).

3.3.5.1.1 *Evidence linking demyelinating lesions to pain syndromes*

3.3.5.1.1.1 Lesion location

Most authors assigned lesions as the likely cause of pain syndromes by anatomical location.

3.3.5.1.1.2 Temporal correlation between lesion and pain syndrome

Relatively few investigators further studied the age or evolution of the lesion in relation to the pain syndrome by use of either serial imaging, or intravenous contrast (used to highlight acute demyelinating lesions).

Please see Table 12 (below) for further details.

Table 12: Location of demyelinating lesions linked to pain syndromes in identified studies

Study	Pain syndrome or location	Reported Lesion Location	Basis of association (Anatomical, Serial imaging, Contrast enhancement)
Spinal cord Tosi 1998	Radicular	Cervical (C5-C6) dorsal root entry zone and posterior horn	A, S
Alstadhaug 2007	Headache (type not defined)	Posterior part of the upper cervical spinal cord	A, S
Burkey 2010	Upper limb pain	Posterior columns from C2 to C4	A
Hellwig 2006	Painful dysaesthesia at thoracic level and/or below	Posterior upper thoracic spinal cord; cord lesions at the level of C1, C4/5, T3 (two cases)	A, C
de Santi 2009	Occipital neuralgia	Right antero-lateral spinal cord at C2; C1, C2, C3 and T1-T2; C2-C3 lesion (three cases)	A, S, C
Marchettini 2006	Back, leg, flank or abdominal pain	Spinal cord location of the lesions assumed; MRI was used to exclude other causes of pain (five cases)	n/a
Brain Andrade 2012	Painful stereotyped involuntary posturing movements of the left upper limb	Pyramidal tract lesions (cerebral peduncle, internal capsule and corona radiata)	A, S, C
Bentley 2002	Painful third nerve palsy (pupil-involving)	Midbrain adjacent to right third nerve fascicle	A, S
Donat 2011	Cluster-like headache	Right dorsal pons	A
González-Quintanilla 2012	Cluster-tic	Left and right trigeminal root inlet and main sensory nucleus in the brainstem	A, S
Tanei 2010	Facial pain (non-TN)	Right dorsal pons and medulla oblongata	A
Haas 1993	Headache (type not defined)	Periaqueductal gray	A, S, C
Liu 2008	Probable TAC with allodynia and other symptoms	Right lateral tegmentum of the lower pons	A, S

Leandri 1999	TAC	Root entry zone of the trigeminal nerve on the right	A
Gentile 2007	Cluster headache/TAC with sensory symptoms	Left brachium pontis	A, S
Meaney 1995	TN (unilateral or bilateral)	Root entry zone of both trigeminal nerves (one case out of seven cases described)	A
Nakashima 2001	TN	Left trigeminal root entry zone (one case out of five cases described)	A
Fragoso 2007	Migraine without aura	Brainstem (two cases)	A
Cordella 2009	TN	Trigeminal root entry zone (five cases)	A
Pichiecchio 2007	TN	Trigeminal root entry zone bilaterally and enhancement of trigeminal nerves	A,C
Vilisaar 2006	SUNCT	Anterior pons, right cerebral peduncle and medulla (one case)	A
Abbreviations A – anatomically plausible lesion; S – serial imaging demonstrating emergence or disappearance of plaque in line with clinical pain syndrome; C – contrast enhancing plaque; n/a = not applicable; TN – trigeminal neuralgia; TAC – trigeminal autonomic cephalalgia; SUNCT – short-lasting unilateral neuralgiform headache with conjunctival injection and tearing; MRI – magnetic resonance imaging			

3.3.6 Treatment of pain

In seven of the studies (20.6% of total), although neuroimaging was used to study pain syndromes in MS, the main focus of the study was an invasive pain treatment. These studies addressed microvascular decompression for trigeminal neuralgia (TN) (185, 195, 198), CNS stimulation (175, 192, 194), and intrathecal administration of steroid (202). These interventions or their efficacy are not further discussed here, as this was not the stated objective of the systematic review.

3.3.7 Further literature identified by search update

Two studies were identified by re-running search strategies, as described.

Lincoln and colleagues, 2016 (212)

Lincoln and colleagues published an educational case report, describing a young man presenting with multiple discrete episodes of multifocal demyelination. The differential was felt to lie between multiphasic ADEM (acute disseminated encephalomyelitis) and MS. A diagnosis of MS was made. Headache was reported as part of his presentations, however this was accompanied by several other symptoms, and was not attributed to any specific demyelinating plaque (212). Headache is a well-established clinical feature of ADEM, and diagnostic difficulty may occur in distinguishing multiphasic ADEM from MS (213). Regardless of the application of a diagnosis of MS, this report does not therefore add significantly to the above conclusions.

Seixas and colleagues, 2016 (214)

Seixas and colleagues carried out a case:control study of 23 people with MS, with and without chronic central neuropathic pain (12 with pain, 11 without, mixed phenotype of relapsing remitting, secondary progressive and primary progressive MS). Subjects with and without pain were matched at the group level for gender, age, disability (EDSS (158)) and disease duration. All had definite MS by McDonald criteria (131). Neuropathic pain questionnaire scores were reported to be consistent with diagnosis of neuropathic pain.

Subjects underwent clinical assessment as well as structural MRI and resting state functional MRI imaging.

Relative to subjects without pain, subjects with pain reported higher levels of depressive symptoms (but not fatigue or anxiety). Structural MRI (analysed using Voxel Based Morphometry, as well as segmentation and volume measurement of subcortical structures (215)) did not show a statistically significant difference between the groups, after correction for multiple comparisons. Trends towards differences in cortical volume (reported as density, though a modulated VBM protocol was used) between groups were seen at the 5% significance level without correction for multiple comparisons. These were interpreted as reflecting trends towards higher cortical density in orbitofrontal and frontal polar cortices, among other locations, in the group without pain.

Default Mode Network connectivity to the caudate and nucleus accumbens was found to be decreased in the pain group. This was interpreted as suggesting alteration in reward networks in the group with chronic pain. White matter lesion volume and location was not reported.

3.4 Discussion

This systematic review is the first to collate and synthesise existing imaging studies of pain syndromes in adults with multiple sclerosis. Please see the thesis Discussion (page 271) for a full discussion of the study findings and methodology, and relevance to the wider field.

In summary, findings included that neuroimaging studies of pain in MS are relatively low in number, and of variable design and quality. Some relatively rare pain syndromes (including Trigeminal Neuralgia) were the focus of a majority of studies. Other, more common, pain syndromes were less frequently studied. Significant methodological issues relating to study design, execution and reporting were identified.

Investigators using different study methodologies have reached differing conclusions regarding the neuroradiological correlates of specific pain syndromes in MS. Methodologically higher-quality studies were however less likely to report positive associations of lesion location to the presence of headache, or of chronic central neuropathic pain (154, 176).

Despite, therefore, the prevalence and impact of pain in MS, the insight into pain mechanisms currently afforded by neuroimaging studies remains limited.

The current evidence does support the hypothesis that focal demyelinating lesions are sometimes associated with the occurrence of specific pain syndromes in MS, in some cases. Study methodology has not, however, always been sufficient to further explore any association. Several studies have not found a clear association of lesion location with the occurrence of specific pain syndromes, and it is possible that MRI-visible lesion location does not explain a significant proportion of the burden of pain syndromes in MS.

There is considerable opportunity to advance our mechanistic understanding of MS-associated pain, and thus its therapy, through future research.

Chapter 4 **Clinical, behavioural and cognitive associations of neuropathic limb pain in adults with multiple sclerosis.**

4.1 Introduction

In this chapter, the clinical and neuropsychological assessment undertaken as part of a prospective case:control study of adults with and without neuropathic limb pain are discussed.

Please see the Introduction (page 1) for a detailed discussion of background, aims and hypotheses.

4.2 Methods

4.2.1 Ethics Committee Review

All procedures described were reviewed and approved by the West of Scotland Research Ethics Committee (study reference 13/WOS/0094).

4.2.2 Participant Recruitment

Study groups: principal groups

There were two principal study groups.

4.2.2.1.1 *MS pain group*

This group comprised people with confirmed relapsing remitting multiple sclerosis, who experienced chronic neuropathic pain in one or more limbs (termed here “MS pain group”)

4.2.2.1.2 *MS controls*

The second was of people with confirmed relapsing remitting multiple sclerosis, who did not experience chronic pain (termed here “MS controls”).

4.2.2.1.3 Subgroup: MS participants without adjuvant analgesics

Where appropriate, and where discussed below, exploratory post-hoc comparisons of the above groups included only subjects who were not administered adjuvant analgesics.

A third study group was established to allow testing of sensory examination techniques in healthy volunteers:

4.2.2.1.4 Healthy controls for sensory testing only

These participants underwent only quantitative sensory testing, and recording of their current medication.

Matching of groups

The MS neuropathic pain group, and the MS control group were matched for age and gender, at the group level.

During recruitment of the MS control group, subjects with non-painful sensory disturbance were preferred if possible. Priority was given to matching for the above variables.

The healthy control group was matched with the MS participant groups for age and gender.

Participants with MS:

Inclusion and exclusion criteria were designed with the intention of identifying a cohort of patients with relapsing remitting multiple sclerosis, who experienced clinically definite neuropathic pain in at least one limb, and of at least one month's duration.

4.2.2.1.5 *Inclusion criteria*

The following inclusion criteria were applied for people with multiple sclerosis:

- Definite diagnosis of multiple sclerosis, by McDonald criteria (76, 131)
- Relapsing Remitting disease course
 - Confirmed by referring clinician/team,
 - And on review of the patient's clinical notes,
 - Or on focussed patient history (if necessary).
- Physically able to undertake the study (including MRI, see Chapters Five and Six)
 - Physical assistance was available for entering/leaving MRI scanner, where needed.
- For people with pain:
 - clinical diagnosis of definite neuropathic pain in at least one limb
 - of at least one month's duration
- Able to provide consent
- Able to communicate freely in English language, including by use of a translator if needed.

4.2.2.1.6 *Exclusion criteria*

The following exclusion criteria were applied for people with multiple sclerosis:

Clinical Associations of Pain in MS

- Lacking capacity to provide informed consent for study participation, in the opinion of the researcher taking consent.
- Receiving “strong” opiates
 - Defined according to step three of World Health Organization (WHO) analgesic ladder (216)
- Confirmed dementia or severe cognitive deficit, previously recorded and likely to negatively influence the patient’s ability to undertake the study protocol, and/or provide informed consent.
- Known contraindication to MRI.
 - For the purpose of this study, pregnancy was considered a contraindication to research MRI imaging.
 - Clinical notes were reviewed, and patients questioned about known contraindications to MRI.
- Course of steroids within the last month
- Clinically confirmed relapse within the last month
 - Participants were excluded if a prior diagnosis of MS relapse within the last month had been made at time of first study visit.
- Unstable or severe psychiatric disease which in the opinion of the lead researcher (PF) would likely impair the patient’s ability to tolerate the study protocol.
 - Patients with psychotic or delusional disorders were excluded from study participation.
 - Patients with a known diagnosis of depression or anxiety were not excluded from the study, as long as the diagnosis was felt to be

secure, and appropriate management and/or review was in place, on review of the clinical notes.

4.2.2.1.7 Medication

It was not felt to be in keeping with ethical practice to ask patients to stop their adjuvant analgesics prior to study participation. In addition, previous investigators suggested that study participants might well opt not to stop their analgesic medications (214).

Patients receiving strong opiates were excluded (see below) because these might impact adversely on ability to complete cognitive assessments.

Healthy controls (for sensory testing only)

Healthy controls were recruited from advertisements in the University, and University staff.

Healthy controls were excluded from participation if

- They had a known history of any neurological disorder
- They had a known history of a chronic pain disorder
- They had used analgesia within the preceding 24 hours

4.2.3 Data acquisition

All data described in this chapter were acquired in a single visit to the Anne Rowling Regenerative Neurology Clinic.

Patients were seen in a clinic room. Patients were routinely given a break during these assessments, and encouraged to ask for and take more breaks, as necessary.

RM acquired neuropsychological data for a subset of the subjects. All other data was acquired by PF. Standardised data entry forms were used where applicable.

Neuropsychology assessment were carried out after other assessments, and after a patient break, in all cases.

4.2.4 Study Measures

Demographics

Core demographic data gathered included subject age, gender, duration of MS, duration of neuropathic pain (for subjects with pain) and current medication.

Age (years) and gender were recorded according to participant self-report, and recorded as a continuous variable and binary variable respectively.

Duration of MS (in years) was recorded according to time elapsed since diagnosis. This measure was used, rather than time since first symptom, as aetiology of reported first symptoms could not be confirmed in retrospect. Disease duration was recorded to the nearest 0.5 years (6 months), and was checked against clinical records, where participants were uncertain.

Duration of neuropathic pain (years) was determined by participant self-report, and recorded to the nearest 0.5 years (ie 6 months).

Medication

Current medication was related by participants, and checked against clinical records where participants were uncertain.

Full listing of all medication (and dosing where known) is presented.

For the purposes of statistical analysis, current use of the following groups of medication was recorded as a binary variable (Table 13).

Table 13: Classification of medication consumption

medication type	example	comments
Weak opiates	dihydrocodeine	participants taking strong opiates (WHO class III) are excluded from study participation
Any adjuvant analgesic	Any adjuvant analgesic including tricyclic antidepressants, gabapentin, pregabalin, SNRIs, Carbamazepine	
Gabapentinoid	Gabapentin or Pregabalin	
Tricyclic Antidepressants	Amitriptyline, Nortriptyline	
Any antidepressant	Any antidepressant of any class	Whether or not typically used for analgesic effects
Baclofen		

Study Instruments: Pain

4.2.4.1.1 Clinical diagnosis of neuropathic pain, in study pain group

The following steps were carried out to ensure recruitment of subjects experiencing clinically definite neuropathic pain, affecting one or more limb, of at least a month's duration.

- Existing secure clinical diagnosis of neuropathic pain made at multiple sclerosis clinic, and/or pain clinic.
- Confirmation of pain of at least one month's duration, affecting at least one limb. Based on clinical interview by trained neurologist (PF).
- Confirmation of neuropathic nature of pain, based on clinical interview by a trained neurologist (PF). The interview took into account
 - Pain descriptors
 - Distribution of pain
 - Timecourse of pain

Clinical Associations of Pain in MS

- Other sensory symptoms in affected area and elsewhere.
- Exclusion of other causes of pain (such as peripheral neuropathy or nerve root impingement) based on clinical interview and examination.
- No imaging or other investigation (eg neurophysiological assessment) was mandatory in order to rule out other aetiologies of a pain syndrome within the study, although some study participants had undergone such assessment for clinical reasons prior to study referral.

4.2.4.1.1.1 Self-report Leeds Assessment of Neurological Symptoms and Signs questionnaire: role in clinical diagnosis of neuropathic pain.

The sLANSS questionnaire score was recorded, but was not required to make a diagnosis of neuropathic pain. No instrument is validated specifically to differentiate neuropathic and non-neuropathic pain in people with MS. The only scale validated for MS neuropathic pain is the Neuropathic Pain Scale, which is felt to be most valuable in evaluating treatment effects (217) (not directly relevant in the current study). The sLANSS instrument has however been used in several studies (9, 218).

There is clinical evidence that some neuropathic pain may be “missed” by screening questionnaires, which are not designed for the purposed discussed. Thus the sLANSS is used to define the extent of neuropathic nature of their pain in a similar fashion to previous studies (Gwilym et al (219)). Please see below (Self Report Leeds Assessment of Neuropathic Symptoms and Signs (sLANSS).for more details.

4.2.4.1.2 *Compatibility of clinical definitions of neuropathic pain, with previous research definitions of neuropathic pain*

Relevant research definitions for the diagnosis of neuropathic pain to the recruited cohort post-hoc were applied (see “results”) in order to compare the definitions used above, to international guidelines on definition of neuropathic pain. In particular, application of relevant IASP and EFNS guidelines (4, 217) was examined.

4.2.4.1.2.1 European Federation of Neurological Sciences (EFNS) criteria, 2010

Cruccu and colleagues (217) suggest 3 criteria for definition of neuropathic pain

1. history –
 - a. character and distribution in accordance with neuropathic pain
 - b. Relevant lesion or disease in nervous system probably responsible for pain
2. Clinical examination reveals negative or positive sensory signs relevant to underlying disease and lesion
3. Further diagnostic tests document specific underlying neurological disease, or confirm sensory lesion within pain distribution

4.2.4.1.2.2 International Association for Study of Pain; Neuropathic Pain Special Interest Group 2008 (IASP NeuPSIG)

Treede and colleagues (4), suggested that the probable or definite presence of neuropathic pain could be assessed by the combination of three main criteria (two of these with sub-criteria):

1. Presence of pain
2. History
 - a. Pain distribution neuroanatomically plausible; and
 - b. History suggests relevant lesion or disease
3. Confirmatory tests

- a. Negative or positive sensory signs, confined to innervation territory of the lesioned nervous structure
- b. Diagnostic test confirming lesion or disease explaining neuropathic pain

For a diagnosis of “definite neuropathic pain” the authors suggest that all of the above criteria are met. For a diagnosis of “probable neuropathic pain” the authors suggest that criteria 1, 2a) and 2b); and *either* 3a) or 3b) are met.

4.2.4.1.3 *Pain severity within pain group*

The Brief Pain Inventory(220) (short form) was used to measure pain severity, as well as pain interference. The BPI has been validated in non-malignant pain. A sample of 440 outpatients with chronic pain of non-malignant aetiology was used for validation. These included patients referred from neurology and physical rehabilitation specialties (221).

Within the BPI, four estimates of pain severity are available (“worst pain in last 24 hours”, “least pain in last 24 hours”, “average” pain, and “pain right now”). In this study the pain severity index (PSI) was used. The PSI is simply calculated by a sum of these four pain severity ratings (222, 223). This technique was chosen to reflect as wide an assessment of participants’ pain experience as possible.

4.2.4.1.4 *Assessment of lack of pain, in study control group*

Because pain is a universal human experience, study aims included to exclude participants with “significant” pain from the control arm of the study. It was not possible to exclude participants with no experience of pain at all. For the purposes of this study, and on discussion among the study team, a question based on phrasing in the Brief Pain Inventory (221, 222) and incorporating definitions of acute and chronic pain to exclude marked acute or chronic pain was used. While some studies of chronic pain define chronicity at pain duration over 6 months (224), a more stringent threshold was employed in this study.

All control group participants were presented the following question, with pre-amble. A forced-choice “yes/no” answer was required.

All control group participants answered “no” to the following question:

"Throughout our lives, most of us have had pain from time to time (such as minor headaches, sprains, and toothaches).

Have you had pain other than these every-day kinds of pain within the last 24 hours, or do you have a problem with pain which has lasted for more than 3 months?"

Study Instruments: Clinical assessment and symptoms

Instruments used in this study were selected according to the following criteria. An iterative process was followed, so that, if possible, an instrument meeting criterion one was used. If this was not possible, an instrument meeting criterion two was used, and so on:

1. Validated in studies of multiple sclerosis
2. Validation in studies of neurological or other disease
3. Widely used in studies of MS.

The following criteria were also met where possible:

Relevance to guidelines for instrument choice in studies of pain interventions reported in the peer-reviewed literature (124).

Relevance to patient reported preferences for measures relevant to pain, reported in the peer-reviewed literature (123).

In the view of the investigating team, could be carried out by study participants within a reasonable timeframe, and without undue burden on study participants. For a more detailed discussion of burden on study participants, see Chapter 7: Participant Experience.

4.2.4.1.5 Expanded Disability Status Scale

The Expanded Disability Status Scale (EDSS) is a clinician-administered scale, generating a score ranging from 0 (normal neurological status) to 10 (death) in half-point increments. The score is generated from a neurological examination, which may require 10 minutes or longer. The lower values of the EDSS measure specific impairments on “functional systems” elicited by clinical examination. Higher scores are heavily dependent on walking ability (158).

It is validated in multiple sclerosis (158). Internationally, it is the most widely used tool to measure multiple-sclerosis related disability (225). The EDSS, however, has

been criticised as being heavily examiner-dependent. All EDSS examinations described in this study were carried out by a single researcher (PF), having received prior recognised and standardised training in the administration of this instrument, and with several years' experience of administering this instrument in a clinical and research environment.

4.2.4.1.6 *Hospital Anxiety and Depression Scale*

The Hospital Anxiety and Depression Scale (HADS) (40) is a brief instrument designed to elicit symptoms suggestive of anxiety and depression. Administration requires 2-3 minutes in most cases. It was initially developed in a general medical population. It has been validated in MS (157) and has been used in a number of studies of people with MS.

For the purposes of this study, separate anxiety and depression scales were generated. A threshold score of eight on the depression subscale has been reported as providing a sensitivity of 90% and specificity of 87.3% for major depression in people with multiple sclerosis (using the Structured Clinical Interview for DSM-IV as a gold standard) (157).

The same threshold score of eight on the anxiety subscale has been reported as providing a sensitivity of 88.5% and specificity of 80.7% for generalized anxiety disorder, in the same study, and using the same gold standard comparator (157).

Use of the HADS meets recommendations for inclusion of measures of emotional function from the IMMPACT collaboration and the IMMPACT collaboration's patient survey on useful measures (123, 124) .

4.2.4.1.7 *Short Form 36 Quality of Life Scale*

The Short Form 36 scale (SF36) (12) is an instrument designed to elicit specific aspects of quality of life. It is very widely used internationally and is used in interventional trials in MS (226).

Completion of the SF-36 takes around ten minutes. It generates eight subscales namely physical functioning, role limitations due to physical health, energy/fatigue, pain, general health, emotional well-being, role limitations due to emotional

problems, and social functioning. Although the SF 36 is widely used, it has been criticised for possible underestimation of mental health problems (227). Floor and ceiling effects have been reported for certain subscales, and in the UK population the use of summary scales has been questioned (228). Shorter versions including the SF-12 are available.

SF-36 was chosen for use in this study because of its wide use, relative ease of use by patients, validation in MS, and inclusion of a pain measure.

4.2.4.1.8 *Fatigue Severity Scale*

The fatigue severity scale (FSS) is a simple self-administered instrument which takes 1-2 minutes to complete for most subjects. It yields an overall estimate of fatigue severity. It has been validated in people with multiple sclerosis (229) and is widely used in studies of multiple sclerosis (230).

The mean fatigue severity score in Krupp et al's original study was 4.8 from a maximum of seven (in a sample of 25 people with progressive subtypes of MS, and EDSS scores ranging from 3.0 to 6.5). Cronbach's alpha was 0.81 (229).

Measurement of fatigue meets recommendations by the IMMPACT collaborators to measure emotional functioning and physical function (FSS questions cover both), and recommendations by patients with chronic pain to measure fatigue (123, 124).

4.2.4.1.9 *Pain Catastrophizing Scale*

The Pain Catastrophizing Scale (PCS) has been validated in adults with pain conditions including low back pain, fibromyalgia (231) and other medical conditions (232). It has been extensively used in multiple sclerosis (59).

Throughout this thesis, the UK spelling of "catastrophising" is used to refer to the concept of pain catastrophising. The USA spelling of "catastrophizing" is used to refer to the original spelling of the PCS.

Pain catastrophising has been described as an exaggerated negative "mental set" brought to bear during painful experiences (58). Participants are asked to what extent they agree with a range of statements, which include "I worry all the time whether

the pain will end”, “I feel I can’t go on” and “It’s awful and it overwhelms me” (233). The pain catastrophizing scale is further discussed in the Introduction to this thesis (1.1.4.1.9).

4.2.4.1.10 *Pittsburgh Sleep Quality Inventory*

The Pittsburgh Sleep Quality Inventory (PSQI) is a self-rated questionnaire which assesses sleep quality and disturbances over the preceding month. Therefore, the data relies largely on self-report.

Additional collateral history from a bed-partner can be added to this questionnaire. This was not used in this study because of practical difficulties in obtaining this information, and limited relevance to the study objectives.

Seven component scores (subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of medication for insomnia, and daytime dysfunction) are generated, as well as a sum global score. It was developed in healthy subjects and “poor sleepers” (the latter with depression or sleep disorder). Global score > 5 was reported as yielding a diagnostic sensitivity of 89.6% and specificity of 86.5% in distinguishing “good” and “poor” sleepers (234).

It has been widely used in studies of MS (235) though is not specifically validated in MS. Assessment of sleep addresses recommendations by the IMMPACT collaboration and pain patients (123, 124).

4.2.4.1.11 *Self Report Leeds Assessment of Neuropathic Symptoms and Signs (sLANSS)*

The self-administered sLANSS instrument estimates the extent of the neuropathic nature of pain, and is completed within about 2 minutes. It comprises self-report of specific symptoms, as well as basic examination carried out by the participant on themselves (for instance sensation to pressure within a pain area).

It has been validated in patients attending outpatient pain services for the purpose of binary distinction between neuropathic and non-neuropathic pain (162). It is not validated in MS, though has been used in studies of pwMS (13, 218).

It is used in this study as an estimate of the extent of neuropathic symptoms (219), rather than to make a binary distinction between neuropathic and non-neuropathic pain. The latter distinction is made by clinical assessment (see above: Self-report Leeds Assessment of Neurological Symptoms and Signs questionnaire: role in clinical diagnosis of neuropathic pain.).

Study instruments: Neuropsychology

4.2.4.1.12 Overview

Please see below (Table 14) for an overview of these instruments, and duration of assessment.

The instruments themselves are then discussed individually, and the specific neuropsychological functions tested by each instrument are described (see Table 15 for summary)

Table 14: Overview of neuropsychology instruments, and duration of administration

Test	Approximate Duration (mins)
BICAMS cognition battery for multiple sclerosis (50), includes Symbol Digit Modalities Test (oral form)(236), California Verbal Learning Test-II (54), Brief Visuospatial Memory Test –Revised T1-3(237)	15
Letter fluency (letter S), and restrained fluency (letter T, four letters)(238, 239)	2
Reverse digit span, letter: number alternation (238)	4
Hayling sentence completion task (238)	5
Delis-Kaplan card sorting test (240)	10
Elevator test with distraction (241)	10

4.2.4.1.13 Brief International Cognitive Assessment for MS (BICAMS)

The BICAMS neuropsychological assessment battery (50) comprises three separate tests which together aim to generate an assessment of verbal memory (immediate and delayed), visual memory and processing speed.

The BICAMS battery lasts around 15 minutes, though there is a requirement for a 25 minute gap to allow assessment of delayed verbal recall. The scales included in the BICAMS battery were chosen (50) for reliability, validity and sensitivity (to MS-

related cognitive dysfunction), as well as ease of application, with a wider aim of standardising international monitoring of cognitive dysfunction in MS.

4.2.4.1.13.1 BICAMS: verbal memory

Verbal memory is assessed by the California Verbal Learning Test-II (54) which includes two components, the first of which tests immediate and the second of which delayed verbal memory. The participant is read a list of 16 nouns. They are asked to repeat as many of these as possible, and the number recalled is recorded. This is repeated until the list has been read, and recall attempted, 5 times in total. Maximum score is $5 \times 16 = 80$.

Delayed recall is tested by asking the participant to recall the same list after a 25 minute interval. The CVLT-II has been validated against a range of imaging measures of MS disease activity, as well as in differentiating employed from unemployed people with MS (50).

4.2.4.1.13.2 BICAMS: visual memory

Visual memory is assessed by the Brief Visuospatial Memory Test – revised T1-3 (237). The participant is shown a 2x3 matrix of 6 simple abstract figures for 10 seconds. The figures are then removed, and the subject is asked to draw these from memory on a sheet of blank paper.

This process is repeated until the figures have been shown, and recall attempted, a total of 3 times. Scoring is by accuracy of recall of the figure shape, and location in the 2x3 matrix. Maximum score = $2 \times 6 \times 3 = 36$. In common with other instruments in the BICAMS battery, scores on the BVMT have been correlated to a variety of imaging measures of MS disease activity (50).

4.2.4.1.13.3 BICAMS: processing speed

Processing speed is tested by the Symbol Digit Modalities test (SDMT) oral form (236). The participant is given a standardised array of abstract shapes, consisting of 9 separate random shapes repeated at random across several rows.

A key is presented which links each shape to a number (1-9). The subject is asked to translate each symbol to the corresponding number, in order, as quickly and as

accurately as possible. A sensitivity of 82%, and specificity of 60% for MS-related cognitive impairment are described (242), and the SDMT has been validated against a variety of imaging measures, as well as employment status (50)

4.2.4.1.14 *Phonemic verbal fluency (letter fluency)*

Verbal fluency is included as a measure of concept generation. Phonemic verbal fluency (letter fluency) (generation of words starting with a particular letter, in contradistinction to semantic verbal fluency, which tests generation of words within a particular category, such as animals) was used (62, 64).

The participant is given instructions to generate as many words as possible starting with a single letter, with the exception of names, numbers or places. The number of words generated in one minute is recorded.

This task was first carried out with the letter S. The task was then repeated with the letter T, with the subject asked to generate only four-letter words. Letter fluency is relatively easy to administer. It is widely used as a measure of concept generation, “energization” (defined as a process of initiating and sustaining a response) and language (63, 238, 239). It has been described as a sensitive marker of early MS-related cognitive impairment (239, 243).

In lesion and clinical studies, phonemic letter fluency has been demonstrated to be linked to a variety of cortical localisations which are also linked to descending modulation of pain (see chapter 5).

4.2.4.1.15 *Delis-Kaplan card sorting test*

The Delis-Kaplan card sorting test is derived from the Delis-Kaplan Executive Function System (D-KEFS) (244), and is also part of the Minimal Assessment of Cognitive Function in MS (MACFIMS) battery (245). The card-sorting test comprises administration of two tests, each using their own set of pre-printed cards. The test is relatively complex and requires approximately 15 minutes.

Each of the two sets of cards comprises six cards. Each card is different from the others, and is distinguished by shape, size, colour, patterns, and words printed on the card. The participant is asked to sort the six cards into two sets of three based on

common features, and to explain the rationale for sorting the groups in this way. The participant is then asked to continue sorting the cards into new paired groups of three, for a total of four minutes.

The groups described by the participant are matched against a pre-prepared list (for example “describes animals” vs “describes means of transport”; “writing in italic font” vs “writing in plain font” and so on). The card sorting test is regarded as a mixed test of concept formation and suppression, with each new concept requiring to be superseded by a new one. It is validated in multiple sclerosis, scores correlate to MRI measures of disease activity, and it has been shown to discriminate between people with and without MS, and employed and unemployed pwMS. In particular, pwMS made fewer correct “sorts” as well as making more perseverative errors, in comparison to healthy controls.(240).

The scoring described here assesses the number of correct group allocations (“sorts”), and the recognition of correct sorts when shown to the participant by the examiner. Perseveration was not examined.

4.2.4.1.16 *Hayling sentence completion task*

The participant is firstly asked, during a dummy run, to generate words which correctly complete a sentence. During the test itself, the instruction is modified so that the participant is asked to generate words which are not related to the word which should complete the sentence. The score allocated to the participant is highest where the word is unrelated to that expected. It is regarded as a combined test of verbal concept inhibition and generation (238). Administration takes around 2 minutes. In the context of this study, the Hayling task is viewed as testing similar functions to the Delis-Kaplan test.

4.2.4.1.17 *Elevator test with distraction (from Test of Everyday Attention)*

The participant is asked, during a dummy run, to count the number of identical tones played on a pre-prepared audio file. During the test itself, additional high-pitched tones are added.

The participant is asked to ignore the high-pitched tones (“distractions”), and only to count the low-pitched tones (246). Administration of the test takes around 10 minutes. The test is regarded as a test of auditory attention, attentional switching and cognitive flexibility. The TEA, of which this test is part, has been tested in MS and has been found to have reasonable ecological validity, ie relevance to real-world impairments (247).

4.2.4.1.18 *Reverse digit span*

During the reverse digit span test, the participant is read a list of numbers, and asked to read them back, without reference to written material, in reverse. The list of numbers starts at length of 2, and increases until the participant fails twice to recount the correct order. The reverse digit span (“digit span backwards”) is often administered as part of neuropsychological batteries such as the Wechsler Memory Scale III (248) and Wechsler Adult Intelligence Scale IV (55).

It is thought to test a variety of functions. These include short term memory, however it is also thought to require focussed attention and manipulation, and is often thought to measure executive abilities, more than storage and rehearsal (64).

Both of the above tests are included in the Edinburgh Cognitive and Behavioural ALS Screen (238) and are regarded as tests of concept generation and inhibition, as well as working memory particularly in the case of the reverse digit span (249).

4.2.4.1.19 *Number:letter sequencing*

During the number:letter alternation test, the participant is asked to continue a pattern of alternating numbers and letters, each increasing stepwise. The examiner reads 1:A, 2:B, 3:C and the participant is asked to continue this pattern, until they are stopped at the letter “O”. This test is included in the ECAS as a combined executive and working memory test (238). It is conceptually similar to the “Trail Making Test B” (250) which asks participants to trace a path between alternating numbers and letters (1-A-2-B...). Executive control is felt to be required for set-switching between

sequential numbers and letters, including inhibition of the currently irrelevant task-set (251), and the trail-making test is widely used to assess set-shifting ability (64).

Table 15: Overview of putative functions tested by individual neuropsychology tests

		Functions tested							
		Verbal Memory		Visual Memory	Processing Speed	Concept Generation	Concept Suppression	Set shifting and cognitive flexibility	Attention
		Immediate	Delayed						
BICAMS	CVLT	+	+						
	BVMT			+					
	SDMT				+				
Phonemic Fluency						+			
Phonemic Fluency (constrained)						+	+		
DKEFS						+	+	+	
Hayling							+	+	
TEA								+	+
Reverse Digit Span		+				+		+	+
Numb:letter sequence		+					+	+	
<p> BICAMS = Brief International Cognitive Assessment in MS CVLT = California Verbal Learning Test BVMT = Brief Visuospatial Memory Test SDMT = Symbol Digits Modality Test DKEFS= Delis Kaplan Executive Function System card sorting task Hayling = Hayling Sentence Completion task TEA = Task of Everyday Attention auditory task with distraction Numb:letter sequence = number/letter sequence </p>									

Study instruments: assessment of sensory symptoms

4.2.4.1.20 *Distribution of pain and sensory symptoms*

4.2.4.1.20.1 Participants with neuropathic pain

Each subject marked the distribution of their pain on a standardised diagram of the human body, included in the Brief Pain Inventory (221). Front and back views were completed. For each subject, an electronic copy of this map was created by using Microsoft Paint, for both front and back.

A symptom distribution map for the pain group was then created by reading the individual files into Matlab, summing them, and dividing by the number of subjects included. Data were output as “.eps” vector images.

Previous studies have used individual subject symptom maps (176), a computer drawing program to produce probability maps (252) or bespoke computerised software including computerised patient interface (253).

4.2.4.1.20.2 Participants without neuropathic pain

Identical procedures to the above were carried out, with the exception that distribution of non-painful sensory symptoms, rather than painful sensory symptoms, were marked. Where the participant did not have sensory symptoms, the symptom map for that participant was left blank.

4.2.4.1.20.3 Comparison of symptom distribution in groups with and without pain

The symptom distribution maps shown were compared visually for distribution of symptoms.

Sensory assessment

A focussed brief quantitative sensory testing protocol was devised, specifically targeted towards clinical signs which could suggest the presence of deficient descending pain modulation systems.

Procedure: Quantitative Sensory Testing (QST)

The following tests were administered (Table 16) to the affected limb, and the upper sternum in the midline:

Wind-up ratio was defined as the ratio of average NRS (numerical rating scale) pain score 0-10 for 10 repeated pinprick stimuli administered at intervals of approximately one second, to NRS pain score 0-10 for a single pinprick stimulus. Where denominator of the ratio was 0 (i.e. where subjects reported a single pinprick as non-painful), the windup ratio was not calculated (in accordance with previous literature) (254, 255) and this data was recorded as not available.

Table 16: Targetted Quantitative Sensory Testing

	Equipment	Equipment supplier	Relevant variables	Outcome measure
Single pinprick (affected limb, central upper chest)	Neurotips	Owen Mumford, UK		NRS 0-10
Windup ratio in limb (affected limb, central upper chest)	Neurotips	Owen Mumford, UK		Wind-up ratio.
Temperature-controlled cool roller	Rolltemp	Somedic, Sweden	25 degC	Cool allodynia: Present/absent
Temperature-controlled warm roller	Rolltemp	Somedic, Sweden	40 degC	Warm allodynia: present/absent
Brush stroke	SenseLab Brush-05 (calibrated)	Somedic, Sweden	approx. 100mN	Dynamic Mechanical Allodynia: Present/absent

In addition, all subjects with neuropathic pain completed the sLANSS instrument in order to assess the extent of neuropathic nature of their pain, at the time seen (256).

Clinical Associations of Pain in MS

4.2.5 Analysis

Data anonymization

Each subject was allocated a four-digit subject ID code, using a computerised number generator (Microsoft Excel). All study data was marked with this ID code. With the exception of consent forms (which were stored separately from other study data), patient identifiable data were not present on paper copies of questionnaires/instruments.

Data storage

Subject data was stored in an individual folder in a locked cupboard, and identified only by 4-digit study ID. Consent forms were stored separately. Anonymised questionnaire data were scanned, saved electronically and backed up on secure University of Edinburgh servers.

Data marking and curation

Clinical assessment data were marked either prior to scanning, or after scanning when the electronic copy of the record was used.

Where marking of the clinical data was complex (227, 234), spreadsheets were generated in Microsoft Excel, and used to calculate scores and subscores. To confirm appropriate marking within Excel spreadsheets, a random sample of questionnaire data were marked separately by hand and by spreadsheet, and results cross-checked.

4.2.5.1.1 Analysis data format and software

Data was recorded in a comma separated value (.csv) spreadsheet file in Microsoft Excel. The spreadsheet was read into R (v3.1.2) implemented in R Studio in Windows 7. Where additional R packages were required, these are specified below.

4.2.5.1.2 Data distribution and statistical tests

For continuous/pseudo-continuous data, data distribution was assessed for Gaussian distribution by inspection of data histograms and Quantile-Quantile (QQ) plot (137, 257, 258).

Where distribution was felt to be Gaussian, mean and standard deviation are reported as summary measures, and Students t-test used to test significance level. Where

distribution was felt to be non-Gaussian, median and interquartile range are reported as summary measures. Wilcoxon rank-sum tests (also known as Mann-Whitney U test) was used to test significance level. No data transformations were used. (137, 257, 258).

For binary variables, a 2x2 table was generated. The Chi-squared test was used for larger samples, and the Fisher exact test for smaller samples (where overall total of the table was less than 20, or where the overall total was between 20 and 40, and the smallest of the four expected numbers was less than 5 (137)).

Significance was assigned at the 5% level, unless otherwise stated. All statistical tests were two-tailed. Correction for multiple comparisons is discussed below.

4.2.5.1.3 Missing data

Missing data was recorded and is discussed where relevant. No imputation was carried out.

4.2.6 Overall analysis strategy

Comparison of subjects with MS, with and without neuropathic limb pain

The principal comparison described in this thesis is between people with MS, with (n=31) and without (n=16) neuropathic pain.

The following analyses are presented:

- Demographics and core data
- Medication
- Psychological and affective morbidity (including depression, anxiety, fatigue and catastrophising)
- Cognitive evaluation: Brief International Cognitive Assessment (BICAMS)
- Cognitive evaluation: executive function
- Sensory evaluation: Quantitative Sensory Testing (QST)
- Sensory evaluation: anatomical distribution of symptoms

Clinical Associations of Pain in MS

Correlates of pain severity in people with MS and neuropathic limb pain

Furthermore, the above variables were explored as correlates of pain severity were explored within the group with neuropathic pain (n=31), where possible given available data.

Type I error and correction for multiple comparisons

In data tables, statistical significance at the 5% significance level before correction for multiple comparisons is indicated. Furthermore, statistical significance at the 5% significance level after correction for multiple comparisons using a straightforward Bonferroni procedure (137) is indicated.

The role of medications

Administration of medication was recorded as described above (Table 13).

. Use of medications between groups was compared as described above.

In post-hoc exploratory analyses, comparison of patients with MS with and without neuropathic pain, where subjects are not receiving any adjuvant analgesic, is also presented.

4.3 Results

4.3.1 Participant Recruitment

People with MS (with and without neuropathic pain)

Participants were recruited between 5th August 2013, and 28th July 2015.

A total of 93 referrals were made to the study. 68 people were referred for inclusion in the group with neuropathic limb pain, and 25 for inclusion in the control group without neuropathic pain. Of these 93 referrals, 92 were made from the Lothian regional MS service based at the Anne Rowling Clinic. One referral was made from the regional pain service for South East Scotland. This patient was not included in the study (because of a diagnosis of secondary progressive MS). Therefore all patients included in the study were referred from the regional MS service. Referrals were made either by the treating clinician, or MS specialist nurse.

Potential participants were not included either because of meeting explicit study exclusion criteria, or because they were not contactable. Reasons for non-inclusion frequently coexisted in a single patient. Primary reasons for study exclusion are shown below (Table 17).

Table 17: Primary reasons for exclusion of potential participants with MS (decreasing frequency)

Primary reason for exclusion		Number of potential participants excluded
Contact	Uncontactable, either at first or subsequent contacts	12
Pain	Pain had settled, symptoms were not described as pain by participant, or cause of pain was uncertain	11
Competing Commitments	Including family, work, travel	10
SPMS	Previously diagnosed, or clinically likely on review of records	5
Active psychiatric problems	Precluding participation	3
Specific contraindication to MRI	Claustrophobia	3
	Known medical contraindication	1
Communication	Not fluent in English, and declined interpreter	1

4.3.2 Application of research definitions of neuropathic pain to study cohort

The two research definitions examined (see Methods: 127) use similar definitions of neuropathic pain (4, 217). All subjects described pain in a neuroanatomically plausible location. All had an existing diagnosis of multiple sclerosis (relapsing-remitting) and had had relevant confirmatory tests to confirm this diagnosis according to contemporaneous guidelines (76).

IASP criteria

31 of 31 subjects (100%) included in the pain group of this study met all criteria listed, and can therefore be categorised as describing “definite neuropathic pain” on the basis of the IASP classification system.

EFNS criteria, 2010

All subjects (31 of 31, 100%) included in the pain group of this study meet all criteria listed, and therefore can be categorised as describing “definite neuropathic pain” on the basis of this classification system.

4.3.3 Overview of participant medications

An overview of participant medications is presented for reference.

Please see below (Table 18)

.

Table 18: Participant Medication

Thesis: Pain in Multiple Sclerosis

Study ID	Non-analgesic medication	Antidepressant other than analgesic	Adjuvant Analgesics	GBP/PGB	SNRI	CBZ	Paracetamol/ NSAID/ weak opiate	MS Disease Modifying Therapy	Baclofen
	STUDY GROUP: HEALTHY VOLUNTEERS (Sensory testing only)								
0193	none								
0238	Citalopram, Lisinopril, Bendrofluazide, Fexofenadine, Seretide, Ventolin	Y							
1272	none								
1491	none								
1573	Clozapine, Diazepam, Lactulose, Loratadine, Lorazepam, Macrogol, Mirtazepine	Y							
1602	Sumatriptan (last used 2/52 previously)								
1883	HRT								
2283	Thyroxine								
2844	none								
3064	Salbutamol, Beclomethasone								
3337	none								
3478	none								
3583	none								
3740	Chlopheniramine								
3833	Ventolin, Citalopram	Y							
3842	Oral Contraceptive Pill								
4168	none								
5233	none								
5270	citalopram	Y							
5568	Topical antifungal								
5820	none								
6371	Oral contraceptive Pill, Limocycline								

Thesis: Pain in Multiple Sclerosis

6471	none								
6703	Mirtazepine, Oral Contraceptive Pill	Y							
6838	Spiriva, Ventolin, Seretide								
6983	Levothyroxine								
8105	None								
8196	Lansoprazole								
8872	None								
9484	none								
9782	none								
9840	none								
STUDY GROUP: MS PAIN									
0104			AMI 50mg nocte						
0326							Paracetamol PRN, Ibuprofen PRN	Beta-Interferon	
0785	none								
0885	Flavoxane 1tab od, Fluoxetine 40mg od	Y		PGB 300mg bd				Fingolimod	Baclofen 60mg/24hrs
1976	Amantadine, Tamsulosin, Clonazepam		AMI 40mg nocte	GBP 1800mg/24hrs			Ibuprofen (occasional)	Beta interferon	
2398	Oral Contraceptive Pill			GBP 300mg nocte				Natalizumab	
2399	Vitamin D, Multivitamin							Glatiramer	
2515	Lansoprazole, Simvastatin, Lisinopril, Folic Acid, Thiamine, Sertraline	Y		PGB 200mg/24h				Beta-interferon	Baclofen 40mg/24h
2742	Amantadine, Fluoxetine 20mg od, Oral Contraceptive Pill, Erythromycin, Thyroxine	Y	Nortriptyline 10mg nocte	GBP 1200mg/24h			Tramadol 100mg od	Glatiramer Acetate	
3051			AMI 30mg nocte	GBP 1800mg/24h			Dihydrocodeine 30mg PRN	Fingolimod	
3216	Nasal Decongestant		AMI 10-20mg nocte						

Thesis: Pain in Multiple Sclerosis

3317	Salbutamol, Venlafaxine 75mg od							Dimethyl Fumarate	
3449	Atenolol, Oral Contraceptive, Vitamin D, Sumatriptan (not received for several week)		AMI 20mg nocte	GBP 900mg/24h				Beta-Interferon	
4161							Co-codamol 30/500 PRN, approx. 180mg codeine/24h		
4250	Cetirizine, Lansoprazole, Bendroflumethiazide, Amlodipine, Thyroxine,			PGB 300mg/24h			Co-codamol, strength unknown, PRN. Ibuprofen 200mg prn	Glatiramer Acetate	
4269	Ventolin, Seretide								
4731			AMI 10mg nocte				Paracetamol 2-3 grams/24h, Aspirin + caffeine (2 per week)		
4824	Eye drops (steroid, cyclopentilate), Antihistamines, Vitamin D, Vitamin C		AMI 50mg/nocte	GBP 900mg/24h					
5028	Fluoxetine 30mg, Lactulose, Hyoscine, Omeprazole, Loperamide, Methylcellulose	Y		GBP 600-900mg/24h			Paracetamol approx. 2g/24h PRN , Etoricoxib 60mg 3/week		Baclofen 10mg/24h
5877	Ferrous Sulphate						Ibuprofen 600mg/week		
6216	Sertraline, Methylcellulose, Oral Contraceptive Pill	Y		GBP 2700mg/24h				Glatiramer Acetate	
6255				PGB 200mg/24h	Duloxetine 60mg		Paracetamol PRN with interferon	Beta-interferon	
6281	Oral Contraceptive Pill, oral acyclovir							Beta-interferon	

Thesis: Pain in Multiple Sclerosis

7157					Duloxetine ?60mg		Co-codamol 30/500 up to qds (PRN)	Fingolimod	
7397	Stemetil, Diazepam 2mg prn		AMI 25mg nocte	GBP 2500mg/24h			Co-codamol 30/500 (PRN)		
8244	Alendronate, Evening Primrose Oil, Vitamin D, anti-oxidant supplement, cranberry supplement						Paracetamol and Ibuprofen PRN infrequently with interferon	Beta- interferon	
8314	Citalopram,	Y	AMI 75mg nocte	Preabalin 300mg/24h				Fingolimod	
8477	Lisinopril, Bendroflumethiazide			PGB 450mg/24h			Paracetamol PRN occasional		
8500	Omeprazole, Gaviscon,		AMI 30mg nocte	PGB ?300mg/24h			Co-codamol 8/500 1 tab infrequently. Ibuprofen infrequently	Beta- interferon	Baclofen dose unclear
9069	Simvastatin 40mg, Candesartan 4mg			GBP PRN					
9494	Inhalers, Oral Contraceptive Pill, Vitamin D							Beta- interferon	
STUDY GROUP: MS CONTROLS									
0735			AMI 75mg nocte					Dimethyl Fumarate	
1279	Hydroxycobalamin, Omeprazole, Vitamin D,		AMI 20mg nocte				Aspirin (concurrently with DMF)	Dimethyl Fumarate	
1294	Solifenacin, Ventolin, Sildenafil PRN							Dimethyl Fumarate	
1337	Oral contraceptive pill					Tegretol Retard 400mg bd	Paracetamol PRN	Natalizumab	
2344	Vitamin D, Hydroxycobalamin							Dimethyl Fumarate	
3286	Fluoxetine 20mg od, melatonin	Y						Beta- interferon	
3724	Oral Contraceptive Pill								

Thesis: Pain in Multiple Sclerosis

3800	Mirtazepine 45mg nocte, longterm Amoxycillin, Multivitamins, Oxybutynin,	Y	AMI 100mg nocte					Beta- interferon	Baclofen 30mg/24h
4038	Thyroxine, Fexofenadine, Adcal D3, Citalopram, Vesicare	Y							
4360								Glatiramer Acetate	
7819	Loratadine, nasal decongestant spray							Fingolimod	
8532	Vitamin D, antihistamine							Dimethyl Fumarate	
8869	Venlafaxine 375mg/24h							Beta- interferon	
9042								Dimethyl Fumarate	
9513	Vitamin D							Dimethyl Fumarate	
9814	Aspirin 75mg od,						Occasional Ibuprofen, occasional Paracetamol	Dimethyl Fumarate	
<p>NSAID = Non Steroidal Anti Inflammatory Drug PRN = as required GBP = Gabapentin PGB = Pregabalin SNRI = Serotonin and Noradrenaline Reuptake Inhibitor CBZ = Carbamazepine</p> <p>Medication doses are given where available Where name of medication was not available (for instance purchased by patient), class of drug is indicated</p>									

4.3.4 Associations of the presence of pain: comparison of MS subjects with and without neuropathic pain

Demographics and matching

Participants with and without neuropathic limb pain are closely matched for age and gender (see Table 19). Groups are also balanced for disability, disease duration, years of full time education. Three participants in the pain group used codeine-containing medications (none in the control group) though this difference did not attain statistical significance at the 5% level (Fisher Exact test). Use of disease modifying therapy (DMT) was somewhat more common in the control group, though this difference did not attain statistical significance at the 5% level (Chi-Squared test).

Use of adjuvant analgesics was significantly more common in the group with neuropathic pain, consistent with expectations. Note that a significant minority of the control group received these medications for indications other than pain. Indications for these medications, as related by patients, was recorded and included insomnia.

Table 19: Demographics and Medication in Participants with and without neuropathic pain

COMPARISON OF SUBJECTS WITH AND WITHOUT NEUROPATHIC PAIN				
DEMOGRAPHICS and MEDICATION				
		Control (no pain) n=16	Neuropathic pain n=31	"p" value
Gender (% female)		81.2	80.6	1.00
Age (years) (median, IQR)		42.50 (33.00 to 52.25)	41.0 (38.0 to 52.0)	0.50
EDSS (median, IQR)		1.75 (1.0 to 2.12)	2.00 (1.50 to 3.02)	0.23
Disease duration (years) (median, IQR)		7.75 (3.37 to 13.62)	7.50 (5.00 to 13.00)	0.73
Years full time education (years) (median,IQR)		15.00 (13.00 to 18.50)	15.50 (12.00 to 17.75)	0.34
Pain Severity (range 0-40) (median, IQR)		NA	17.00 (10.00 to 22.00)	NA
Medications	Weak opiates (%) (codeine-containing medication) (note that no participants received strong opiates)	0 (0%)	3/31 (9.7%)	0.5412
	Any adjuvant analgesics (%) (gabapentin/tricyclic/other)	3/16 (18.75%)	22/31 (70.97%)	0.0015 *
	Any antidepressant medication	6/16 (37.5%)	18/31 (58.1%)	0.30
	Tricyclic Antidepressant	3/16 (18.75%)	11/31 (35.5%)	0.32
	Gabapentinoid	0/16 (0%)	17/31 (54.8%)	0.0001 *
	Baclofen	1/16 (6.25%)	4/31 (12.9%)	0.65
	MS DMT (%)	14/16 (87.5%)	19/31 (61.2%)	0.094
<p>* = statistical significance at 5% level</p> <p>MS DMT = multiple sclerosis disease modifying therapy (including interferon and other therapy)</p> <p>No correction for multiple comparisons applied</p> <p>SD=Standard Deviation</p> <p>IQR=Interquartile range</p>				

Emotional and affective morbidity

4.3.4.1.1 Symptom scores

Subjects with neuropathic pain demonstrated significantly more emotional/affective comorbidity measured by scores for depression, fatigue, and catastrophising, even after Bonferroni correction for multiple comparison (Table 20 below). Anxiety scores were higher in the pain group ($p < 0.05$ not corrected for multiple comparisons).

Table 20: Psychological Comorbidity in participants with and without neuropathic pain

	Control (no pain) n=16	Neuropathic pain n=31	95% CI of difference between samples	“p” value
Depression (HADS)	1.50 (0.00-6.00)	5.00 (2.00 – 8.50)	0.99 to 5.00	0.005134 **
Anxiety (HADS) (median, IQR)	5.50 (2.00-9.25)	9.00 (6.00-12.00)	0.99 to 6.00	0.02662 *
Fatigue (FSS) (median, IQR)	33.00 (26.75-43.75)	51.00 (43.50-56.50)	4.00 to 22.00	0.003371 **
Catastrophising (PCS) (median, IQR)	8.50 (0.75-15.00)	16.00 (12.50 – 25.00)	4.00 to 15.00	0.003593 **
* = statistical significance at 5% level ** = statistical significance at 5% level after Bonferroni correction for multiple comparison HADS = Hospital Anxiety and Depression Scale FSS = Fatigue Severity Scale PCS = Pain Catastrophizing Scale				

4.3.4.1.2 Depression and Anxiety meeting diagnostic criteria

11/47 (23.4%) of all subjects met diagnostic criteria for major depression (157). No subjects in the control group met these criteria, however 35% of the pain group met these criteria. Subjects whose scores met these cut-offs were more commonly found in the pain group (Table 21).

26/57 (55.3%) of all subjects met diagnostic criteria for generalized anxiety disorder (157). Subjects meeting these criteria were common in both control and pain groups. There was no statistically significant difference in prevalence of subjects meeting generalized anxiety disorder criteria in comparison of the two groups (see Table 21).

Clinical Associations of Pain in MS

Table 21: subjects meeting cut-offs for major depression, and generalized anxiety

	Control (no pain) n=16	Neuropathic pain n=31	“p” value
Depression (HADS) (number, %)	0/16 (0%)	11/31 (35.48%)	0.0085 **
Anxiety (HADS) (number, %)	7/16 (43.75%)	19/31 (61.29%)	0.40
* = statistical significance at 5% level ** = statistical significance at 5% level after Bonferroni correction for multiple comparisons HADS = Hospital Anxiety and Depression Scale			

Cognitive evaluation: Brief international Cognitive Assessment in MS (BICAMS)

Performance on immediate verbal memory and visual memory was worse in the group with neuropathic pain, than the control group, to a statistically significant degree (5% significance level after correction for multiple comparisons), and for delayed verbal memory to the 5% significance level (without correction for multiple comparisons)..

Processing speed (SDMT) was not worse in the pain group, to a statistically significant degree (see Table 22).

Table 22: Memory and Processing Speed in participants with and without neuropathic pain

	Control (no pain) n=16 (median, IQR)	Neuropathic pain n=31 (median, IQR)	95% CI of difference between samples	“p” value
CVLT-II (word list)	60.50 (53.25-67.25)	45.00 (41.50-58.50)	-20.00 to -3.00	0.01077 **
CVLT-II (delayed recall)	15.00 (12.50-16.00)	11.00 (8.00-14.00)	-5.00 to -1.00	0.01466 *
BVMR-R	32.50 (30.00-34.25)	28.00 (22.50-31.00)	-7.00 to -1.00	0.00931 **
SDMT	62.50 (61.00-65.25)	57.00 (47.00-64.00)	-15.00 to 2.00	0.1275
<p>* = statistical significance at 5% level ** = statistical significance at 5% level after Bonferroni correction for multiple comparisons</p> <p>CVLT-II = California Verbal Learning Test-II BVMR-R = Brief Visuospatial Memory Test –Revised T1-3 SDMT = Symbol Digits Modality Test</p>				

Cognitive evaluation: executive function

Measures of letter fluency and inhibition of extraneous (auditory) information were not different in the groups with and without pain, to a statistically significant degree.

Three of four measures of set-shifting or cognitive flexibility generated by the DK-EFS were significantly worse in the group with pain. Performance in the number:letter alternation task was often at ceiling (median score 12 in both groups, of maximum possible 12). Performance in the Hayling test was close to ceiling in both groups. Performance in the reverse digit span task was not significantly different in the pain and control groups (Table 23).

Table 23: Executive Functions in participants with and without neuropathic pain

		Control (no pain) n=16	Neuropathic pain n=31	95% CI of difference between samples	“p” value
Concept generation					
	Fluency, letter S (mean, SD)	17.00 (4.04)	15.00 (4.94)	-4.42 to 1.07	0.2243
	Constrained fluency, letter T (median, IQR)	9.00 (7.00-11.75)	8.50 (7.00-11.00)	-3.00 to 2.00	0.6551
Inhibition of extraneous information					
	Elevator Test with Distraction	9.00 (8.60-10.00)	9.50 (5.75-10.00)	-3.00 to 1.00	0.7086
Set shifting					
	Card Sorting 1	7.00 (6.00-7.00)	5.00 (5.00-6.00)	-2.00 to -1.00	0.0005576 **
	Card Sorting 2	5.00 (4.50-6.00)	5.00 (5.00-6.00)	-1.00 to 1.00	0.8249
	Recognising Card groups 1	24.00 (21.00-24.00)	12.00 (8.00-20.00)	-14.00 to -8.00	6.316 x10 ⁻⁶ **
	Recognising Card groups (2)	20.00 (20.00-24.00)	12.00 (10.00-16.00)	-12.00 to -4.00	0.0001834 **
	Reverse Digit Span	6.00 (6.00-7.25)	7.00 (6.00-8.00)	-1.00 to 1.00	0.5979
	Alternating numbers and letters	12.00 (9.00-12.00)	12.00 (12.00-12.00)	-0.01 to 2.00	0.08504
	Hayling sentence completion	11.00 (10.00-12.00)	11.00 (9.00-11.00)	-2.00 to 6.32x10 ⁻⁶	0.1736
* = statistical significance at 5% level ** = statistical significance at 5% level after Bonferroni correction for multiple comparisons					

Sensory evaluation: Quantitative Sensory Testing**4.3.4.1.3 Group demographics and testing sites**

There was no statistically significant difference in age or gender between healthy volunteers and people with MS, nor between people with MS with or without neuropathic pain. There was no statistically significant difference between site of QST testing (defined as right vs left limb, and upper vs lower limb) between healthy volunteers and people with MS, nor between people with MS with or without neuropathic pain (Table 24).

Table 24: Demographics and testing site in quantitative sensory testing groups

	Healthy volunteer (n=31)	People with MS (ALL) (n=47)	MS without neuropathic pain (n=16)	MS with neuropathic pain (n=31)	“p” value healthy volunteer vs MS	“p” value MS with, vs MS without neuropathic pain
Age (years) (median, IQR)	43.0 (31.0 to 51.0)	41.0 (35.5 to 52.0)	42.5 (33.0 to 52.25)	41.0 (38.0 to 52.0)	0.48	0.50
Gender (count, % female)	23/31 (74.20%)	38/47 (80.85%)	13/16 (81.25%)	25/31 (80.64%)	0.68	1.00
Upper limb tested (count, %)	11/31 (35.48%)	13/47 (27.66%)	4/16 (25.0%)	9/31 (29.0%)	0.67	1.00
Laterality of testing: Right side (count, %)	17/31 (54.84%)	22/47 (46.80%)	5/16 (31.25%)	16/31 (51.61%)	0.13	0.86

4.3.4.1.4 *Comparison of healthy volunteers and people with MS*

Pain score to single pinprick was not significantly different in the healthy volunteer or MS groups.

Wind-up ratio, both in the limb affected by pain (or sensory disturbances) and over the xiphisternum, was higher in patients with MS, than healthy volunteers (5% significance level). There were high numbers of missing data for Wind-Up Ratio, in both healthy volunteers and people with MS. All cases of missing data occurred because of participants scoring a single pinprick as “0” severity.

The presence of allodynia of any type was more frequent in people with MS, than healthy volunteers (5% significance level) though individual measures of allodynia, (cool and warm stimulus, and dynamic mechanical allodynia) were not significantly more frequent in the MS group (Table 25).

Table 25: Quantitative sensory testing in healthy volunteers, and people with MS

		Healthy Volunteer (n=31)	MS (ALL) (n=47)	“p” value
Single pinprick	Single pinprick (median, IQR)	1.0 (0 to 1.25)	0.50 (0.00 to 0.98)	0.57
Wind-up	WUR (limb) (median, IQR)	1.0 (1.0 to 1.0) NA = 11	1.25 (1.0 to 1.63) NA = 24	0.0026 **
	WUR (central) (median, IQR)	1.0 (1.0 to 1.46) NA = 9	1.4 (1.0 to 1.75) NA = 20	0.037 *
	Any WUR greater than one (count, percentage)	7/31 (29%)	22/47 (47%)	0.054
Allodynia	Cold Allodynia (count, percentage)	0/31 (0%)	5/47 (10.64%)	0.15
	Warm Allodynia (count, percentage)	1/31 (0.03%)	5/47 (10.64%)	0.39
	Dynamic Mechanical Allodynia (count, percentage)	0/31 (0%)	4/47 (8.51%)	0.15
	Any Allodynia (count, percentage)	1/31 (0.03%)	8/47 (17.02%)	0.038 *
<p>* = statistical significance at 5% level ** = statistical significance at 5% level after Bonferroni correction for multiple comparisons</p> <p>Limb = testing at affected limb Central = testing at xiphisternum in midline NA = missing data WUR = wind up ratio IQR = Interquartile Range</p>				

4.3.4.1.5 Comparison of participants with and without neuropathic pain in MS

Sensitivity to single pinprick was higher in those with neuropathic pain, than those without. Allodynia (when any type of allodynia was included) was more likely to occur in the pain group than the group without pain. Other differences did not attain statistical significance (Table 26).

There were high numbers of missing data for Wind-Up Ratio. All cases of missing data occurred because of participants scoring a single pinprick as “0” severity.

Table 26: Quantitative Sensory Testing in people with MS, with and without neuropathic pain

		MS without neuropathic pain (n=16)	MS with neuropathic pain (n=31)	“p” value
Single pinprick	Single pinprick (median, IQR)	0.00 (0.00 to 0.50)	1.00 (0.00 to 2.00)	0.019 *
Wind-up	WUR (limb) (median, IQR)	1.00 (1.00 to 1.00) NA = 12	1.50 (1.00 to 2.50) NA = 12	0.07
	WUR (central) (median, IQR)	1.17 (1.0 to 1.38) NA = 8	1.50 (1.22 to 2.00) NA = 12	0.09
	Any WUR greater than one (count, percentage)	4/16 (25%)	18/31 (58.0%)	0.065
Allodynia	Cold Allodynia (count, percentage)	0/16 (0%)	5/31 (16.1%)	0.15
	Warm Allodynia (count, percentage)	0/16 (0%)	5/31 (16.1%)	0.15
	Dynamic Mechanical Allodynia (count, percentage)	0/16 (0%)	4/31 (12.9%)	0.28
	Any Allodynia (count, percentage)	0/16 (0%)	8/31 (25.8%)	0.038 *
sLANSS	Total score (median, IQR)	N/A	14.0 (10.0 to 18.5)	N/A
<p>* = statistical significance at 5% level ** = statistical significance at 5% level after Bonferroni correction for multiple comparisons</p> <p>Limb = testing at affected limb Central = testing at xiphisternum in midline WUR = wind up ratio IQR = Interquartile Range sLANSS = self report Leeds Assessment of Neuropathic Symptoms and Signs</p>				

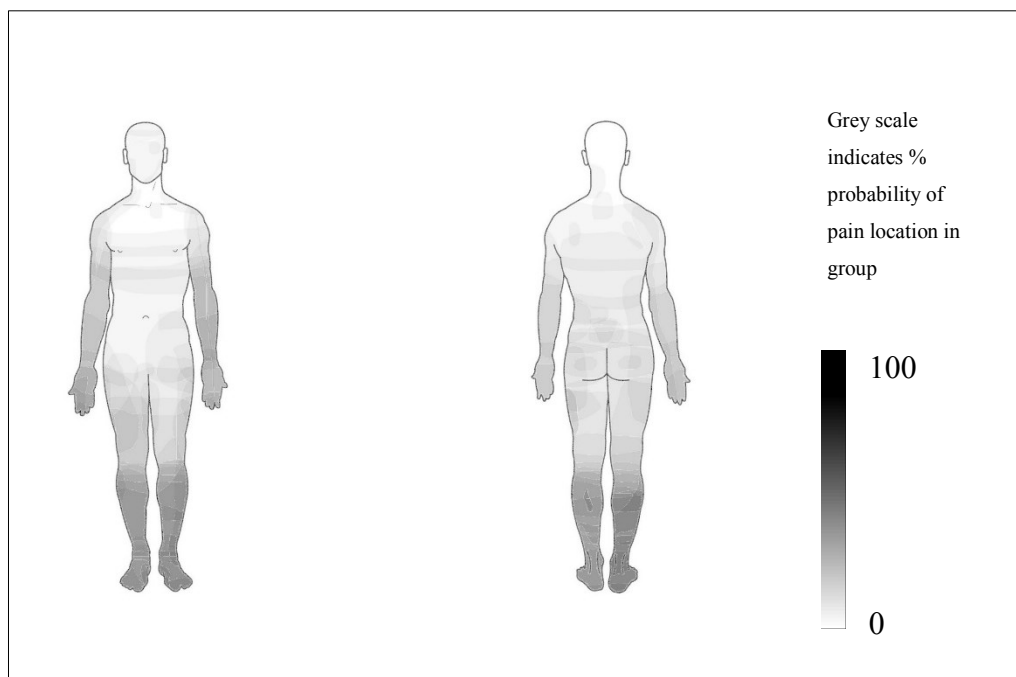
Anatomical symptom distribution

4.3.4.1.6 Neuropathic pain group

Of 31 patients with neuropathic limb pain, all indicated the location of pain on the body diagrams as part of the Brief Pain Inventory.

Distribution of pain symptoms in subjects with neuropathic pain is shown below (Figure 10)

Figure 10: Probability distribution of neuropathic pain (n=31) (JPEG data shown due to memory constraints)



On review of the above distribution maps, probability of pain was highest in the limbs, and in the lower rather than upper limbs. Symptoms appeared symmetrical. Symptoms were also seen in the abdomen and torso, including (in some cases) in a band-like distribution across the chest.

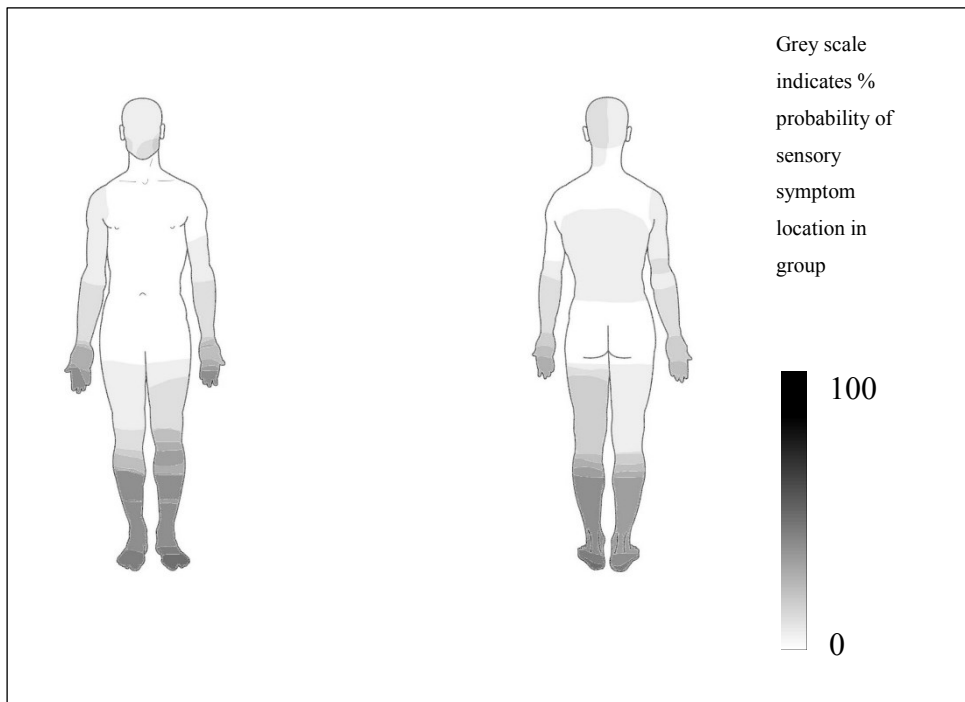
No patient reported symptoms on the anterior upper central chest wall, or on the occiput.

4.3.4.1.7 Group without neuropathic pain

Of 16 patients without neuropathic pain, 14 described neuropathic sensory symptoms, which were not painful. All 14 marked the distribution of these symptoms on the Brief Pain Inventory body map as described.

Distribution of non-painful neuropathic sensory symptoms in all 16 subjects without neuropathic pain is shown below (*Figure 11*).

Figure 11: Probability distribution of non-painful neuropathic sensory symptoms (n=16)(JPEG data shown due to memory constraints)



On review of the above distribution maps, probability of non-painful neuropathic sensory symptoms was highest in the limbs, and in the lower rather than upper limbs. Symptoms laterality appeared symmetrical. Symptoms were also seen in the abdomen and torso, though no patient described a band-like (radicular) distribution on the anterior chest.

No patient reported symptoms on the anterior torso or abdomen, buttocks, or upper posterior chest.

4.3.4.1.8 *Comparison of groups with and without neuropathic pain*

Both groups showed bilateral fairly symmetrical symptoms, mainly affecting the limbs, with a preponderance in the lower limbs. In both groups, the head was occasionally affected. The abdomen and torso appeared to be more frequently affected in the group with neuropathic pain. No formal statistical analysis of difference is currently possible, and the small group size in the control group in particular is noted.

Quality of life and sleep

Overall quality of life (sum SF36 score) was significantly worse in subjects with pain, than those without (median 592.2 vs median 425.2, $p=0.000185$) (Table 27).

All eight quality of life subscale scores reflected worse quality of life in the pain group. Statistical significance at the 5% level was reached for all 4 of the physical subscales, and one of the 4 mental subscales.

Table 27: Quality of life: Comparison of subjects with and without pain

	Control (no pain) n=16 (median, IQR)	Neuropathic pain n=31 (median, IQR)	95% CI of difference between samples	“p” value (Mann-Whitney test)
Quality of life (total score)				
SF36 total score	592.2 (507.6 to 635.1)	425.2 (337.4 to 517.8)	-244.5 to -88.3	0.000185 **
Quality of life subscales: “Physical”				
Physical Functioning	85.0 (61.5 to 95.0)	60.0 (37.5 to 80.0)	-3.5 to -3.2x10 ⁻⁵	0.04 *
Role limitations due to physical health	75.0 (50.0 to 100.0)	25.0 (0.0 to 50.0)	-50.0 to -25.0	0.00049 **
General Health	60.0 (47.5 to 75.3)	40.0 (30.0 to 55.0)	-30.0 to -5.0	0.0175 *
Pain	100.0 (90.0 to 100.0)	45.0 (32.5 to 67.5)	-55.0 to -32.5	1.7x10 ⁻⁷ **
Quality of life subscales: “Mental”				
Emotional well-being	80.0 (58.0 to 92.0)	72.0 (58.0 to 78.0)	-2.0 to 4.5x10 ⁻⁶	0.064
Social functioning	75.0 (59.3 to 100.0)	62.5 (43.75 to 75.0)	-2.5 to 1.6x10 ⁻⁵	0.065
Energy/Fatigue	47.5 (30.00 to 70.00)	35.0 (22.5 to 50.0)	-2.5 to -3.4x10 ⁻⁶	0.037 *
Role Emotional	100.0 (92.92 to 100.0)	100.0 (33.3 to 100.0)	-3.33 to 4.2x10 ⁻⁵	0.09
* = statistical significance at 5% level ** = statistical significance at 5% level after Bonferroni correction for multiple comparisons QoL – Quality of Life SF36 – Short Form 36 Quality of Life instrument (higher values, better quality of life) Significance threshold set at 5%				

4.3.4.1.9 Sleep

Median sum PSQI scores in the MS control group was 6.00 (IQR 3.75 to 7.25).

Median sum PSQI score in the MS pain group was 8.00 (5.50 to 12.00). Sleep was rated as significantly worse in the group with neuropathic pain (p=0.028)

4.3.5 .Subgroup exploration of associations of the presence of pain: subjects not receiving adjuvant analgesics

Demographics and matching

Thirteen MS control patients, and nine MS pain patients, were not receiving any regular adjuvant analgesics.

After exclusion of all subjects receiving adjuvant analgesic drugs, no significant difference was found between subjects with and without pain for gender, age, EDSS, disease duration, years full-time education, administration of baclofen, or administration of MS DMT (Table 28).

Table 28: Exploration of demographics and matching: subjects not receiving adjuvant analgesics

		Control (no pain) n=13	Neuropathic pain n=9	“p” value
Gender (% female)		7/9 (77.8%)	10/13 (76.9%)	1.0
Age (years) (median, IQR)		44.00 (33.00 to 53.00)	42.00 (39.00 to 49.00)	0.61
EDSS (median, IQR)		1.50 (1.00 to 2.00)	1.50 (1.0 to 1.5)	0.53
Disease duration (years) (median, IQR)		9.50 (3.00 to 15.50)	6.00 (5.00 to 10.00)	0.71
Years full time education (years) (median,IQR)		16.00 (13.00 to 18.25)	16.00 (12.00 to 17.00)	0.57
Medication	Baclofen	0/13	0/9	1.0
	MS DMT (%)	11/13	5/9	0.18
* = statistical significance at 5% level MS DMT = multiple sclerosis disease modifying therapy (including interferon and other therapy) No correction for multiple comparisons applied SD=Standard Deviation IQR=Interquartile range				

Psychological and affective morbidity

After exclusion of all subjects receiving adjuvant analgesic drugs, statistically significant differences between those with and without neuropathic pain were found for depression scores, anxiety scores and fatigue scores. Catastrophising scores did not attain statistical significance at the 5% level ($p=0.06$) (Table 29). Directions of effect were the same as observed when all subjects were analysed together (see Table 20).

Table 29: Exploration of psychological and affective morbidity scores: subjects not receiving adjuvant analgesia

	Control (no pain) n=13	Neuropathic pain n=9	"p" value
Depression (HADS)	4.00 (0.00 to 6.00)	8.00 (5.00 to 11.00)	0.013 *
Anxiety (HADS) (median, IQR)	7.00 (2.00 to 9.00)	12.00 (9.00 to 13.00)	0.0098 **
Fatigue (FSS) (median, IQR)	32.00 (27.0 to 40.00)	50.00 (45.00 to 56.00)	0.0134 *
Catastrophising (PCS) (median, IQR)	10.00 (3.00 to 15.00)	19.00 (15.00 to 22.00)	0.060
* = statistical significance at 5% level ** = statistical significance at 5% level after Bonferroni correction for multiple comparisons HADS = Hospital Anxiety and Depression Scale FSS = Fatigue Severity Scale PCS = Pain Catastrophising Scale			

Cognitive evaluation: Brief international Cognitive Assessment (BICAMS)

After exclusion of all subjects receiving adjuvant analgesic drugs, no statistically significant differences were seen between groups with and without neuropathic pain on any measures from the BICAMS battery.

In the cases of CVLT-II (word list), CVLT-II (delayed) and BVMR-R, there was a trend to worse performance in the pain group ($p=0.052$ to 0.078), in the same direction as observed in the overall analysis (Table 22). In the case of SDMT, no trend was observed, in keeping with results in the overall analysis (Table 30).

Clinical Associations of Pain in MS

Table 30: Exploration of BICAMS measures: subjects not receiving adjuvant analgesia

	Control (no pain) n=13 (median, IQR)	Neuropathic pain n=9 (median, IQR)	“p” value
CVLT-II (word list)	61.00 (54.00 to 67.00)	46.00 (41.00 to 72.00)	0.052
CVLT-II (delayed recall)	15.00 (13.00 to 16.00)	11.00 (9.00 to 14.00)	0.078
BVMR-R	33.00 (32.00 to 35.00)	29.00 (25.00 to 31.00)	0.076
SDMT	63.00 (61.00 to 65.00)	64.00 (62.00 to 79.00)	0.35
<p>* = statistical significance at 5% level ** = statistical significance at 5% level after Bonferroni correction for multiple comparisons</p> <p>CVLT-II = California Verbal Learning Test-II BVMR-R = Brief Visuospatial Memory Test –Revised T1-3 SDMT = Symbol Digits Modality Test</p>			

Cognitive evaluation: executive function

After exclusion of all subjects receiving adjuvant analgesic drugs, statistically significant differences (at the 5% significance level) were seen between the groups with and without pain, in three measures of cognitive flexibility (Table 31).

Differences attaining statistical significance were the same as those found to be statistically significant in the overall analysis prior to exclusion of subjects not receiving adjuvant analgesics, and were in the same direction (see also Table 23).

Table 31: Exploration of executive functions: subjects not receiving adjuvant analgesia

		Control (no pain) n=13	Neuropathic pain n=9	"p" value
Concept Generation				
	Fluency, letter S (mean, SD)	17.00 (4.08)	15.00 (4.44)	0.32
	Constrained fluency, letter T (median, IQR)	9.00 (7.00 to 14.00)	7.00 (6.50 to 9.50)	0.50
Inhibition of extraneous information				
	Elevator Test with Distraction	9.00 (9.00 to 10.00)	9.00 (5.50 to 10.00)	0.74
Cognitive flexibility				
	Card Sorting 1	7.00 (6.00 to 7.00)	5.50 (5.0 to 6.0)	0.016 *
	Card Sorting 2	5.00 (5.00 to 6.00)	6.00 (5.00 to 6.25)	0.36
	Recognising Card groups 1	24.00 (23.00 to 25.00)	20.00 (19.00 to 20.00)	0.0046 **
	Recognising Card groups (2)	22.00 (20.00 to 25.00)	18.00 (15.50 to 20.00)	0.049 *
	Reverse Digit Span	6.00 (6.00 to 8.00)	8.00 (6.00 to 9.00)	0.44
	Alternating numbers and letters	10.00 (9.00 to 12.00)	12.00 (12.00 to 12.00)	0.50
	Hayling sentence completion	11.00 (10.00 to 12.00)	11.0 (9.00 to 11.00)	0.47
* = statistical significance at 5% level				
** = statistical significance at 5% level after Bonferroni correction for multiple comparisons				

Sensory evaluation

After exclusion of all subjects receiving adjuvant analgesic drugs, no statistically significant differences were seen between the MS groups with and without neuropathic pain. Trends towards higher rates of allodynia and higher wind-up ratio (as seen in the overall analysis prior to exclusion of subjects receiving adjuvant analgesic drugs) (Table 26) were seen ($p = 0.055$ and 0.071 respectively) (Table 32).

Table 32: Exploration of quantitative sensory testing: subjects not receiving adjuvant analgesics

		MS without neuropathic pain (n=13)	MS with neuropathic pain (n=9)	"p" value
Single pinprick	Single pinprick (median, IQR)	0.00 (0.00 to 0.46)	1.50 (0.38 to 2.00)	0.053
Wind-up	WUR (limb) (median, IQR)	1.00 (1.0 to 1.00) NA = 9	1.50 (1.12 to 1.88) NA = 3	0.071
	WUR (central) (median, IQR)	1.17 (1.00 to 1.38) NA = 5	1.33 (1.25 to 1.50) NA = 4	0.40
	Any WUR greater than one (count, percentage)	4/13 (31.76%)	6/9 (66.67%)	0.19
Allodynia	Cold Allodynia (count, percentage)	0/13 (0%)	1/9 (11.11%)	0.41
	Warm Allodynia (count, percentage)	0/13 (0%)	2/9 (22.22%)	0.16
	Dynamic Mechanical Allodynia (count, percentage)	0/13 (0%)	2/9 (22.22%)	0.16
	Any Allodynia (count, percentage)	0/13 (0%)	3/9 (33.33%)	0.055
sLANSS	Total score (median, IQR)	N/A	16.00 (14.00 to 17.00)	N/A
* = statistical significance at 5% level ** = statistical significance at 5% level after Bonferroni correction for multiple comparisons WUR = wind up ratio IQR = Interquartile Range sLANSS = self report Leeds Assessment of Neuropathic Symptoms and Signs				

4.3.6 Correlates of pain severity

Demographics

Participants with neuropathic limb pain of low and high severities are balanced for age and gender, disease duration, pain duration, and years full time education.

As would be expected pain severity is significantly higher in the “high pain” group.

Disability (EDSS) is also significantly higher in the “high pain” group. Notably EDSS was not significantly different between groups with and without pain, although subjects were not deliberately matched for physical disability at any stage (see above).

Table 33: Demographics and Medication in participants with varying severities of neuropathic pain

COMPARISON OF SUBJECTS WITH LOW- AND HIGH-SEVERITY NEUROPATHIC PAIN				
DEMOGRAPHICS and MEDICATION				
		Low pain severity n=16	High pain severity n=15	“p” value
Gender (% female)		81.25	80.00	1.00
Age (years, sd)		44.50 (9.41)	43.27 (10.98)	0.74
EDSS (median, IQR)		1.50(1.00 to 2.00)	5.50 (2.25 to 5.75)	0.0073 *
Disease duration (years)		6.00(4.75 to 10.75)	10.00 (5.50 to 13.50)	0.36
Pain duration (years)		5.75(2.00 to 6.62)	6.00 (2.75 to 15.00)	0.23
Years full time education (median,IQR)		16.50 (12.75 to 17.50)	13.50 (12.00 to 17.75)	0.51
Pain Severity (range 0 to 40) (median, IQR)		10.50(4.00 to 13.75)	22.00 (19.50 to 24.00)	6.93x10 ⁻⁵ *
Medication:	MS DMT (%)	62.50	60.00	1.00
	strong opiates (%) (WHO level III)	0	0	1.00
	Other opiates (%) (codeine-containing medication)	12.50	6.67	1.00
	Adjuvant analgesics (%) (gabapentin/tricyclic/other)	68.75	73.33	1.00
MS DMT = multiple sclerosis disease modifying therapy (including interferon and other therapy)				
* = statistical significance at 5% level				
No correction for multiple comparisons applied				

Clinical Associations of Pain in MS

Pain severity measures

4.3.6.1.1 *Pain severity: summary statistics*

Median “average” rating of pain severity was 5.00 from a maximum possible of 10 (IQR 3.00 to 6.00). A score of 5 is at the upper end of a previously defined range from 3 to 5 for “moderate” pain in people with multiple sclerosis (259).

Median “worst” pain severity was 7.00 from a maximum possible of 10 (IQR 3.00 to 8.00). A score of 7 is at the upper end of a previously defined range for “moderate” pain in people with multiple sclerosis, which ranged from 5 to 7 (259).

Pain Severity Index (PSI) median was 17.00 from a maximum of forty (IQR 10.00 to 22.00). There are no published definitions of pain severity in MS relating to PSI scores, however a recent study of people with neuromyelitis optica described comparable PSI scores of median 15 (antibody negative patients, some of whom were felt possibly to have opticospinal MS) and 20 (antibody positive patients) (223).

4.3.6.1.2 *Pain severity: correlations between differing BPI measures*

In the MS Pain group, Pain Severity Index (PSI) was highly correlated with all measures of pain severity from the Brief Pain Inventory (Spearman’s Rho 0.86 to 0.93).

The Average Pain measure from the BPI was similarly highly correlated to other measures from the BPI, however to a lesser degree than the PSI (Spearman’s Rho 0.76 to 0.85).

PSI and average pain were less strongly correlated with the pain severity index from the SF-36 (Spearman’s Rho -0.58 for PSI, noting that SF-36 pain scale indicates worse pain with a lower score, so that negative correlation is expected). SF-36 measures pain over the preceding four weeks, in comparison to one week for the BPI. Please see Table 34.

Table 34: Exploration of correlations between pain severity measures (Spearman's Rho , $n=31$)

	PSI	Average pain (BPI)	Worst Pain (BPI)	Least pain (BPI)	Pain now (BPI)	Pain severity (SF36)
PSI	-					
Average pain (BPI)	0.93	-				
Worst Pain (BPI)	0.86	0.85	-			
Least pain (BPI)	0.87	0.76	0.61	-		
Pain now (BPI)	0.93	0.82	0.66	0.87	-	
Pain severity (SF36)	-0.58	-0.55	-0.58	-0.50	-0.50	-
BPI = Brief pain Inventory PSI = Pain Severity Index SF 36 = Short Form 36						

Psychological and affective morbidity

Symptoms of anxiety, fatigue and catastrophising were all significantly positively correlated with pain severity in those suffering from neuropathic pain, after Bonferroni correction for multiple comparisons (Table 35). Symptoms of depression were significantly correlated at the 5% level before correction for multiple comparisons.

Table 35: Psychological and psychiatric comorbidity: correlation with pain severity

	Correlation (Spearman's Rho)	"p" value
Depression (HADS)	0.414	0.021 *
Anxiety (HADS)	0.584	0.00056 **
Fatigue (FSS)	0.60	0.0004 **
Catastrophising (PCS)	0.57	0.0009 **
* = statistical significance at 5% level ** = statistical significance at 5% level after Bonferroni correction for multiple comparisons HADS = Hospital Anxiety and Depression Scale FSS = Fatigue Severity Scale PCS = Pain Catastrophising Scale		

Cognitive evaluation: Brief International Cognitive Assessment in MS (BICAMS)

Verbal memory (immediate or delayed), visual memory and processing speed were not correlated to pain severity at the 5% significance level (see Table 36).

Table 36: Memory and Processing Speed: correlation with pain severity

	Correlation (Spearman's Rho)	"p" value
CVLT-II (word list)	0.035	0.85
CVLT-II (delayed recall)	0.084	0.655
BVMR-R	0.209	0.259
SDMT	0.081	0.672
* = statistical significance at 5% level ** = statistical significance at 5% level after Bonferroni correction for multiple comparisons CVLT-II = California Verbal Learning Test-11 BVMR-R = Brief Visuospatial Memory Test –Revised T1-3 SDMT = Symbol Digits Modality Test		

Cognitive evaluation: executive function

There was no statistically significant correlation of any executive function test with pain severity (Table 37).

Table 37: Executive function measures: correlation with pain severity

		Correlation (Spearman Rho unless otherwise stated)	"p" value
Concept generation			
	Fluency, letter S (mean, SD)	-0.07 Pearson	0.70
	Constrained fluency, letter T	0.056 Pearson	0.80
inhibition			
	Elevator Test with Distraction	0.045	0.82
Set shifting			
	Card Sorting 1	-0.05	0.80
	Card Sorting 2	-0.24	0.21
	Recognising Card groups 1	-0.22	0.25
	Recognising Card groups 2	0.36	0.10
	Reverse Digit Span	-0.11	0.57
	Alternating numbers and letters	0.11	0.54
	Hayling sentence completion	-0.086	0.65

Quality of life and related variables

Overall quality of life (sum SF36 score) was significantly correlated with pain intensity, in those with neuropathic pain.

All eight quality of life subscales (both physical and mental) were significantly correlated with pain intensity, at the 5% significance level (see Table 38, below).

Table 38: Quality of Life: Correlation between pain severity and quality of life measures

	Correlation (Spearman's Rho)	"p" value
Quality of life (total score)		
SF36 total score	-0.65	7.8x10 ⁻⁵ **
Quality of life subscales: "Physical"		
Physical Functioning	-0.41	0.023 *
Role limitations due to physical health	-0.48	0.006 *
General Health	-0.57	0.00088 **
Pain	-0.58	0.00066 **
Quality of life subscales: "Mental"		
Emotional well-being	-0.60	0.00037 **
Social functioning	-0.53	0.0023 **
Energy/Fatigue	-0.47	0.008 *
Role Emotional	-0.54	0.002 **
* = statistical significance at 5% level		
** = statistical significance at 5% level after Bonferroni correction for multiple comparisons		
SF36 – Short Form 36 Quality of Life instrument (higher values, better quality of life)		

4.3.6.1.3 Pain interference

In the MS Pain group, pain severity was significantly correlated with pain interference in all measured activities included in the Brief Pain Inventory (221).

Table 39: Correlations of pain severity with pain interference scores

	Correlation (Spearman's Rho)	"p" value
Pain Interference (total score)		
Pain Interference total score	0.78	2.55×10^{-7} **
Pain interference subscales		
General Activity	0.66	5.90×10^{-5} **
Mood	0.61	0.00028 **
Walking Ability	0.80	5.96×10^{-8} **
Normal Work (includes work outside the home and housework)	0.77	4.31×10^{-7} **
Relations with other people	0.67	3.50×10^{-5} **
Sleep	0.63	0.00017 **
Enjoyment of Life	0.72	5.6×10^{-6} **
* = statistical significance at 5% level ** = statistical significance at 5% level after Bonferroni correction for multiple comparisons SF36 – Short Form 36 Quality of Life instrument (higher values, better quality of life)		

4.3.6.1.4 Sleep

Sum Pittsburgh Sleep Inventory score was significantly related to pain severity, indicating worse ratings of sleep quality with increasing pain severity (Spearman's Rho 0.68, $p=2.14 \times 10^{-5}$).

4.3.7 Participant experience of study

The study protocol was well tolerated. No subjects withdrew from the clinical, behavioural and cognitive assessments. Specific data on participant experience of the study, including clinical, behavioural, cognitive and radiological assessments, was gathered separately and is presented in Appendix: Participant Experience.

4.4 . Discussion

The results described in this chapter suggest that, in the population described, emotional/affective factors are associated with both the presence and severity of neuropathic limb pain in relapsing-remitting multiple sclerosis. Cognitive factors (specifically memory and cognitive flexibility) are associated with the presence of neuropathic pain, but not severity of existing pain.

Please see the thesis Discussion (page 290) for a full discussion of findings, relevance to the wider field and study methodology.

Interpretation of the difference between groups with and without neuropathic pain is limited by the higher prevalence of adjuvant analgesics in the pain group, however a post-hoc exploratory analysis limited only to those not receiving such drugs supports the conclusions of the wider analysis.

Both emotional/affective, and cognitive factors have been repeatedly linked to descending modulation of pain. The described results could be compatible with the study hypothesis of impaired pain modulation, in the population described.

These results, if confirmed in future larger studies, could be relevant to understanding of pain modulatory mechanisms in MS, and could lead to further investigation of how these systems might be impaired (for instance by focal demyelination, or by grey matter atrophy). These results are among the first to explore the cognitive correlates of neuropathic limb pain in MS, and support future consideration of cognitive as well as emotional/affective and sensory aspects of pain in this disorder. From a treatment perspective, these findings could eventually be relevant to development and administration of targeted therapies (57, 260).

Chapter 5 **Structural imaging correlates of neuropathic limb pain in adults with multiple sclerosis**

5.1 Introduction

This chapter describes structural imaging assessments undertaken as part of the prospective study described in Chapter Four. For a full discussion of background, aims, and hypotheses please see the Thesis Introduction (page 1).

Existing studies examining neuroimaging correlates of pain syndromes in MS are described elsewhere in this thesis. Please see Chapter Three (page 90).

5.2 Methods

5.2.1 Structural image acquisition

All MRI imaging was performed on a single Siemens Verio 3Tesla (3T) MRI scanner (Erlangen, Germany) at the Clinical Research Imaging Centre (CRIC), University of Edinburgh.

A 12-channel phased array head coil manufactured by Siemens was used. No scanner upgrades were carried out during the execution of this study.

Imaging was acquired on the same day as completion of the Brief Pain Inventory (261) in all cases. Imaging was acquired in a single session in all cases.

T1-weighted, T2-weighted and FLAIR sequences all acquired 1mm isotropic data (ie with 1 x 1 x 1mm voxels). The following structural imaging was acquired (Table 40):

Table 40: Specifications of structural imaging sequences

Sequence	Specifications	Acquisition time (total 15 minutes, 13s)
T1 MPRAGE (T1-weighted)	field of view 256 x 240 x 160 mm voxel dimensions 1 x 1 x 1mm flip angle 9 degrees TR 2300ms TE 2.98 ms TI 900ms.	5 minutes, 3s.
T2 SPACE (T2-weighted)	Field of view 256 x 256 x 160 mm voxel dimensions 1 x 1 x 1mm TR 3200ms TE 416ms.	4 minutes, 18s
T2 FLAIR	Field of view 256 x 256 160mm voxel dimensions 1 x 1 x 1mm TR 5000ms TE 402ms TI 1800ms.	5 minutes, 52s
TR = Repetition Time TE = Echo Time TI = Inversion recovery time SPACE = “Sampling Perfection with Application optimized Contrasts using different flip angle Evolution” (Siemens proprietary sequence) MPRAGE = Magnetization-Prepared Rapid Gradient-Echo FLAIR = Fluid-Attenuated Inversion Recovery		

5.2.2 General Image Analysis

General preprocessing of imaging data

5.2.2.1.1 *Anonymisation of data*

All imaging data was fully anonymised at source (Julian Sparrow), and subsequently identified only by a computer-generated random 4 digit numerical subject code.

Subject codes were allocated by a random number generator in Microsoft Excel (262).

5.2.2.1.2 *Overview of analysis software*

Various image analysis programs were used, subject to specific requirements. These are further described below. The majority of analyses described use the FSL (FMRIB Software Library) package (Oxford, UK). Software versions used were as follows:

FSL v5.0.1 (105), SPM version 12 (263), Mango Image Viewer version 3.4 (264), Analyze version 11.0 (265), Spinal Cord Toolbox version 2.0 (266) and Matlab 2014a (267).

Analysis was implemented on the BRIC imaging servers (University of Edinburgh, UK) (Dell Poweredge R620 with 32 cores and 96GB RAM, running Scientific Linux 2.6.32-504.8.1.el6.x86_64).

5.2.2.1.3 *Data format*

Imaging data was output from the University of Edinburgh Clinical Research Imaging Centre in DICOM (Digital Imaging and COMmunication) format. DICOM files were converted to compressed Nifti format using Chris Rorden's `dcm2nii` (268). Subsequent conversion to Analyze or Nifti (uncompressed) format, if required, was done with `fschfiletype` (105).

5.2.2.1.4 *Alignment of images to standard orientation*

MPRAGE, T2-weighted and FLAIR images were re-orientated to match the orientation of the MNI152 standard space template, using `fsloreorient2std` (105). Images were viewed in `fslview`.

5.2.2.1.5 *Cropping of neck tissue*

Extraneous neck tissue was manually cropped from MPRAGE, T2 and FLAIR images at the level of the mid-C3 vertebra following visual identification for each individual subject, and using the fslroi tool within FSL. This step was carried out to optimise later preprocessing including brain extraction.

5.2.2.1.6 *Noise reduction*

For voxel base morphometry analyses, and lesion analyses, no smoothing was applied.

For spinal cord analyses described, MPRAGE, T2 and FLAIR images were smoothed prior to further processing using FSL's SUSAN, using the following parameters: “-1 3 3 1 0” ie threshold at 10% of robust range, halfwidth 3mm, 3 dimensional smoothing with local median filter (269).

5.2.3 **Reporting of statistical significance**

5.2.3.1.1 *Whole brain analyses*

Throughout, the 5% significance level, fully corrected for multiple comparisons, is reported in whole-brain analyses. These include analyses of lesion distribution or grey matter volume.

Where appropriate, and where described, the less stringent threshold of $p < 0.001$, uncorrected for multiple comparisons, is reported as previously described in the literature (80).

For the purposes of direct comparison with one previous VBM study of people with RRMS (214), with and without neuropathic pain, VBM results from the comparison of subjects with and without pain were thresholded at the 5% significance level, as used in that study.

5.2.3.1.2 *Region of interest analyses*

Results were assigned statistical significance at 5% significance level, fully corrected for multiple comparisons, in region of interest analyses.

In order to reduce the likelihood of Type 1 error, the less stringent threshold of $p < 0.001$, uncorrected for multiple comparisons, was not reported for region of interest analyses.

5.2.3.1.2.1 Pre-specified region of interest analyses

Grey matter volume: single three-dimensional mask of descending pain modulatory system (see below)

T2 hyperintense lesions: three-dimensional mask of brainstem (see below)

Standard space templates

Throughout this chapter, widely used standard space templates are used. The MNI152 templates at 1mm and 2mm resolution (referred to as MNI152 1mm and 2mm templates) were used. These atlases are derived from 152 T1 structural brain images, averaged together after high-dimensional nonlinear registration into the common MNI152 co-ordinate system (270).

For Voxel Based Morphometry the MNI 2mm template was used, because this is the standard template used for the well-validated FSL VBM protocol, and is recommended by the FSL group for use in this protocol (271). Use of this protocol additionally ensures comparability of results with other experiments.

For lesion analysis used the MNI 1mm template was used, in order to maximise resolution of the lesion probability maps in relation to small structures, specifically T2 hyperintense lesions and the brainstem.

In the latter case, a study-specific template was not used, as use of the widely-recognised MNI template additionally allows exploration of results using probabilistic atlases packed with FSL (105, 116, 272), both for reporting anatomical location of results, and for creating standardised masks (see above).

Anatomical localisation

Where specified, the Harvard-Oxford cortical or subcortical atlases (116, 272), or, in the case of brainstem anatomy, the Duvernoy atlas of the human brainstem and cerebellum (273) have been used.

Creation of three-dimensional masks for region of interest analyses.

Where available, masks were based on the Harvard-Oxford cortical or subcortical structural atlases, packaged with FSL (105, 116, 272). These atlases provide probability maps of structure locations, in MNI 2mm standard space.

Probability maps of structures were saved locally, thresholded at 50% probability (to exclude voxels of <50% likelihood of involvement in the structure) and then binarised using *fslmaths*, to create a three-dimensional mask. All voxels within the mask were assigned the value “1”, and those outside the mask the value “0”.

Masks of the dorsolateral prefrontal cortex were kindly supplied by Dr Katja Wiech, FMRIB centre, University of Oxford. DLPFC masks were created as previously described (65). Briefly, masks were hand-drawn on T1 images based on Brodmann areas 8, 9, 46, 9/46 (approximately corresponding to the superior and middle frontal gyrus) (65).

5.2.3.1.3 *Brainstem mask in 1mm standard space*

The binarised brainstem mask in 2mm standard space created using the above method was transformed to 1mm standard space for use in lesion analyses. FSL’s FLIRT (274) was used, and registration to the standard space template checked visually.

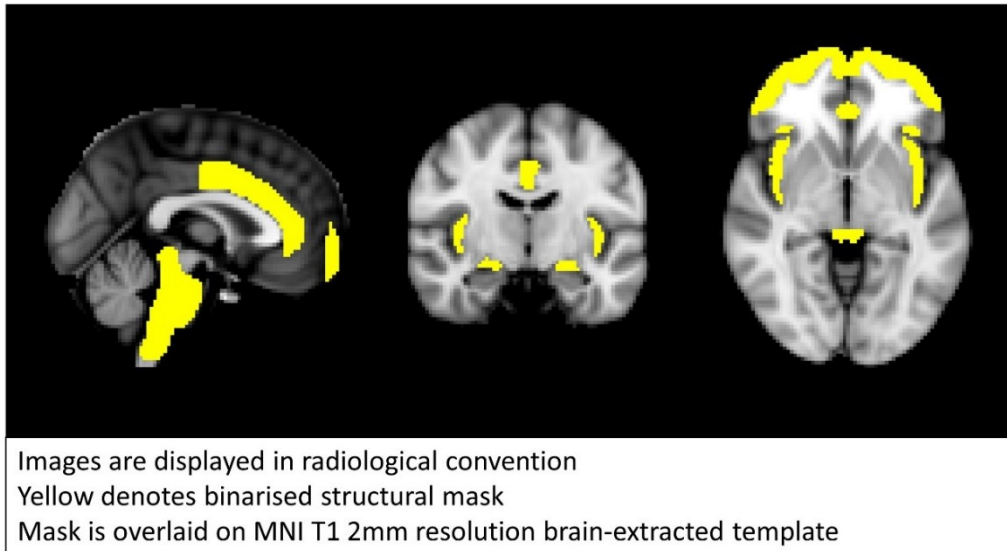
5.2.3.1.4 *Descending pain modulatory system mask*

A single mask was created including the bilateral dorsolateral prefrontal cortex, bilateral insula, brainstem, anterior cingulate cortex, bilateral amygdala, orbitofrontal cortex and frontal pole. These structures were chosen based on published reports of anatomical structures involved in descending modulation of pain. Orbitofrontal cortex and frontal pole were included given the particular focus on any role of cognitive variables including set-shifting (Table 2) (1, 29, 33, 64, 96).

Because of the exploratory nature of this study, masks of the whole brainstem, whole anterior cingulate and whole insulae were used. In other words, analysis of these structures was not further restricted to subregions such as rostral anterior cingulate, anterior insula, periaqueductal gray matter and rostral ventromedial medulla.

This mask is illustrated below (Figure 12: Mask of supraspinal descending pain modulatory system)

Figure 12: Mask of supraspinal descending pain modulatory system used in this study



5.2.4 Intracranial Volume Estimation: comparison of methods

General

Intracranial Volume (ICV) was estimated by manual and automated methods in a subset of 28 study subjects (the entire study sample available at time of this analysis). Manual and automated ICV estimates calculated by these methods were compared by exploratory scatterplots, analysis of correlation and Bland-Altman plots.

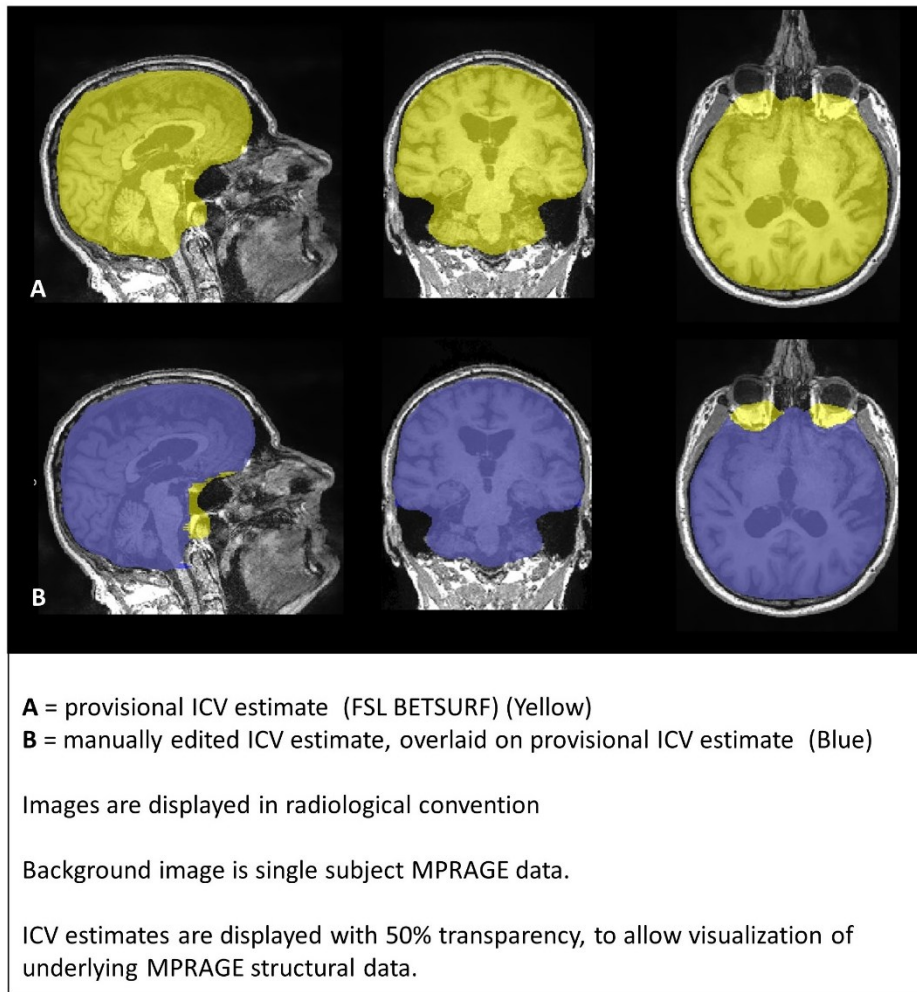
Automated ICV estimation using SPM, and semi-automated ICV estimation were compared. These methods were chosen because ICV estimation in SPM has been reported to be an acceptable alternative to manual segmentation (275). The current study, however, includes patients with differing central nervous system pathology, and uses different acquisitions. Therefore, automatic ICV estimation using SPM, and semi-automated ICV estimation were compared. A comparison of separate automated methods (SPM and FSL) was not carried out as this was not the focus of the current study.

Manual estimation of ICV:

Preliminary intracranial volume (ICV) estimates were calculated using BETSURF, a modification of FSL's Brain Extraction Tool (BET) package (276, 277) using multispectral (T1- and T2-weighted) input. Brain extraction was centred on the approximate centre of each subject's brain (identified visually in FSLVIEW), using the "-c" option within BET. The 'innerskull mesh' estimate output by BETSURF was used as an initial guide to the outer boundary of ICV.

Resultant estimates were then manually edited for accuracy in Analyze v11.0 (265). A trained neurologist (PF, trained by Maria Valdes Hernandez) manually traced ICV for each subject, using native space MPRAGE images. The inferior limit of the ICV was set as the foramen magnum (odontoid peg just visible on axial images). The ICV estimate resulting from BETSURF was edited in contiguous sagittal slices. This process is further illustrated below (Figure 13).

Figure 13: process of manual ICV estimation



The total volume of the resultant manually edited ICV estimate was calculated using `fslmaths`.

Fully automated estimation of ICV

Each subject's smoothed and cropped T2-weighted and FLAIR images were linearly coregistered to each subject's MPAGE image using affine space transformation in FSL's FLIRT (278, 279). A normalized mutual information cost function, with 12 degrees of freedom, was used.

The coregistered images were then subject to multispectral segmentation (using T1-, T2-weighted and FLAIR images) using New Segment in SPM12 (263). Three tissue classes were specified. Tissue volumes were calculated by the "tissue volumes"

utility supplied within the batch editor in SPM. This computes the totals of modulated warped tissue class segmentations (275).

Intracranial volume was calculated by summing grey matter (GM), white matter (WM) and cerebrospinal fluid (CSF) estimates obtained from New Segment implemented in SPM 12 (263, 275). This method has been found to produce estimates of ICV which closely correlate with manual image segmentation, including in subjects with CNS neurodegenerative disease (275).

Comparison of manual and automated ICV estimation

Associations between ICV estimates for each subject were explored using a scatterplot. Correlation was calculated using Spearman's Rho. In order to further investigate mean difference between manual and automated estimates of ICV, a Bland Altman plot was then constructed using the Bland Altman function available from the MethComp package in R (280).

Based on the above findings (see results), further analysis used automated estimates of ICV for the study group as a whole (n=45).

5.2.5 Tissue type segmentation

General description and preprocessing

Volumes of separate tissue classes (Grey Matter, White Matter and Cerebrospinal Fluid) (GM, WM and CSF) were calculated as described above (5.2.4).

5.2.6 Lesion segmentation

Working definition of T2 hyperintense lesions

MS lesions (termed here white matter lesions, or WMLs) were defined as appearing hyperintense in relation to surrounding white matter or grey matter on T2 weighted and FLAIR images, with an irregular or ovoid appearance, and most commonly occurring in the periventricular white matter, juxtacortical and infratentorial regions (76, 281).

Specifically, care was taken to avoid misclassification of enlarged perivascular spaces. In cases where there was any doubt as to whether an MRI hyperintensity represented a WML (in contradistinction to ischaemic lesion, enlarged perivascular space or imaging artefact), the hyperintensity was excluded from analysis.

A semi-automated approach to generating single subject lesion maps was used. This process comprised the following steps:

Independent blinded neuroradiologist review of imaging

An experienced neuroradiologist (Professor Sellar), blinded to patient status with the sole exception of the diagnosis of multiple sclerosis, identified lesion location using each subject's MPRAGE, T2-weighted and FLAIR images in native space. A report was generated and subsequently anonymised.

Provisional automated lesion map generated for each subject

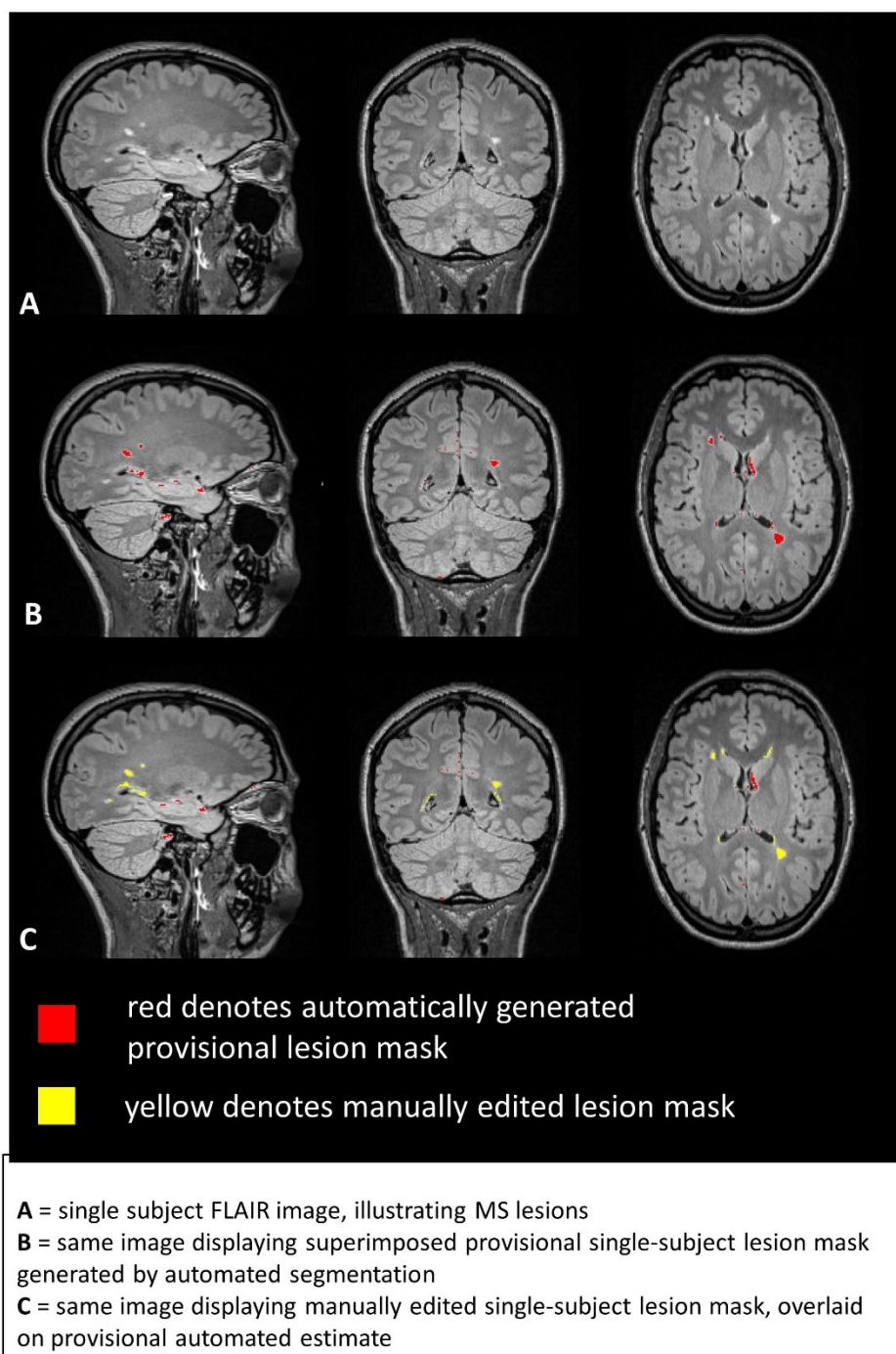
Intensity thresholding of FLAIR images at greater than 2 standard deviations above mean image intensity of the brain parenchyma was carried out using a program developed in-house (Maria Valdes-Hernandez) and implemented in MatlabR2014a (267). Intensity thresholding of the FLAIR images was carried out automatically after applying the ICV mask generated by semi-automated techniques described above.

Creation of manually edited lesion map for each subject

Lesion maps were then edited manually, using each subject's FLAIR image, by a neurologist trained in lesion identification (PF), with simultaneous review of T2-weighted imaging using Mango image viewer (264), and using the existing neuroradiology report in order to minimise misclassification (see above). Each subject's data was identified only by the subject's computer-generated 4-digit subject code. Images were viewed in the axial plane and sagittal planes.

This process is illustrated below (Figure 14). Please note the contrast of manual and automated lesion mask estimates (B and C).

Figure 14: Illustration of semi-automated MS lesion segmentation process



Calculation of lesion volume in native space

Total lesion volume for each subject was calculated in the subject's native space using `fslmaths` in cubic millimetres. In order to take into account possible effects of head size, which was a covariate of no interest, lesion volume was expressed for each subject as a fraction of the intracranial volume (ICV).

5.2.6.1.1 *Calculation of brainstem lesion volume in native space*

In a further exploratory analysis, based on the study hypotheses, lesion volume within the brainstem only was calculated.

A brainstem mask was created as described above (5.2.3.1.3). Subsequently the mask was registered to individual subjects' native space (using the inverse of the nonlinear warp described below), registration checked visually in `FSLVIEW`, and lesion volume within the native space brainstem ROI calculated using `fslmaths`.

Intra-rater reliability of manual lesion segmentation

A sample of 9 study subjects were selected for evaluation of the reproducibility of manual lesion segmentation.

5.2.6.1.2 *Selection of study subjects*

9 study subjects were selected in order to approximate 20% of the total study sample. The subjects were selected at random from the whole study group (including subjects both with and without pain) using their computer-generated subject ID codes, and using the command "sample" implemented in R, $n=9$, no resampling).

5.2.6.1.3 *Calculation of intra-rater reliability*

Using identical methods to those outlined above, binary lesion masks were created without sight of the previous white matter hyperintensity map. The original automated lesion estimation was not repeated.

Lesion volumes in subject native space were calculated as described above. Volumes derived by the first and second manual segmentation processes were calculated using `fslmaths`, and compared using a Bland-Altman plot, and the Intraclass Correlation Coefficient with accompanying 95% confidence interval (implemented in R using the packages 'MethComp'(280), and 'psych'(282), respectively). Time elapsed

(days) between first and second estimates were reported, as short times elapsed could make memory effects more prominent.

5.2.7 Group-level lesion probability maps

Transforming single-subject lesion masks to standard space

Each subject's MPRAGE T1 image was linearly registered to their T2 image using FSL's FLIRT (with 12 degrees of freedom) and a correlation ratio cost function (278, 279).

For purposes of registration, this MPRAGE image was then skull-stripped using FSL's BET, and this skull-stripped image then registered to the Montreal Neurological Institute (MNI152) skull-stripped 1mm template with a nonlinear registration procedure, implemented in FSL's FNIRT (274). Skull-stripped T1 (MPRAGE) images were used for registration according to existing recommendations (274, 278, 279).

The resulting nonlinear transformation matrix was then applied to each individual subject's lesion mask to transform the mask into the space of the MNI152 1mm skull-stripped template. A nearest neighbour cost function was used, to remove the requirement for re-thresholding of binary lesion maps. Masks were reviewed visually after transformation to standard space, to ensure accuracy of registration.

Creating group-level lesion probability maps

Lesion probability maps for each group were created by summing and then averaging individual lesion maps in MNI space. In-house software implemented in Matlab (written by Maria Valdes-Hernandez) was used.

The resulting lesion probability maps represent the percentage probability of lesion location at each voxel in the standard-space image, within the study group. Separate lesion probability maps were created for

- The study group as a whole
- Subjects with neuropathic pain
- Control subjects without neuropathic pain

5.2.7.1.1 *Comparison of group-level lesion probability maps*

Lesion probability maps for each group were firstly compared visually. To firstly explore the difference between group lesion probability, probability maps from each group were simply subtracted using `fslmaths` to create a ‘difference map’ using a method previously described (283).

In order to identify any lesion location which might be statistically significantly different between groups with and without pain, after taking account of multiple comparisons in space, the nonparametric permutation inference tool `Randomise` was used (284) as described in previous work (283).

For each study group, individual subjects’ lesion distribution maps were concatenated in the temporal dimension by using `fslmerge` (105), in order to create a single 4-dimensional ‘image’. An unpaired t-test analysis design was set up using FSL’s GLM GUI, and the concatenated lesion maps then analysed for difference between pain and control groups. `Randomise` was run with 5000 permutations, within a binary mask of the skull-stripped MNI 152 1mm template (distributed with FSL), and using threshold-free cluster extent (TFCE) statistics, with correction for multiple comparisons and 5% significance threshold.

5.2.8 **Voxel Based Morphometry**

Voxel Based Morphometry (VBM) was carried out using FSL VBM (271). T1 MPAGE images were used, having previously removed extraneous neck data using `fslroi` (see above for description 5.2.2.1.5), and carried out lesion filling.

Lesion filling

Each subject’s binary lesion map, created as described above, was linearly transformed from T2 space to their native-space MPAGE image, using a linear transformation in FSL’s `FLIRT` (278). Registration was checked visually.

Automated lesion filling was carried out on each subjects MPAGE images to minimise impact of MS lesions on subsequent tissue-class segmentation.

`Lesion_filling` implemented in FSL (215) was used. Lesion filling has been

demonstrated to reduce misclassification of focal lesions as grey matter and has been implemented in FSL (215, 285) and SPM (286)

VBM processing

VBM processing followed standard protocols. Images were first brain-extracted within the FSL VBM pipeline. Brain extraction was checked manually in all cases. Where brain extraction was not satisfactory, further brain extraction was optimised on a per-subject basis using options within FSL-BET (276) including manual centring (“-c” call). Grey matter was then segmented and registered to the MNI 152 standard space using non-linear registration (274). The resulting images were averaged and flipped along the x-axis to create a study-specific grey matter template which is symmetrical left:right.

All native grey matter images were then non-linearly registered to this study-specific template. Modulation by the Jacobian of the warp field was used to correct for local expansion due to the non-linear component of the spatial transformation.

The modulated grey matter images were smoothed with an isotropic Gaussian kernel with a sigma of 3mm (approximate full-width-half-maximum 7mm). All templates were checked visually.

A voxelwise general linear model was applied using permutation-based non-parametric testing, correcting for multiple comparisons across space. Tests were applied across either the whole grey matter, or the predefined mask of the descending pain modulatory system (as described), specified by the “-m” call within randomise (287). Threshold-free cluster extent testing was used.

ICV (estimated by summing GM, WM and CSF volumes (275, 288)) was included as a nuisance covariate in all VBM analyses. Age was included in all VBM analyses apart from where correlates of pain duration were investigated, because pain duration and age were found to be moderately collinear ($Rho\ 0.41$, $p=0.02$) and non-inclusion of age in this model avoided this source of collinearity (96).

5.2.8.1.1 *Voxel Based Morphometry in SPM*

Voxel based morphometry is widely used in the SPM package as well as the FSL package. Although SPM was used for tissue class segmentation and consequent estimation of ICV, as described above, SPM was not used for the final VBM analysis described.

In particular, estimates from the VBM analysis are output in a format comparable to other analyses in this thesis, which were carried out in FSL. For instance, SPM outputs images in neurological rather than radiological convention. By using FSL VBM, estimates from the VBM analysis could be input into further analyses (such as resting state fMRI analyses) if required.

5.2.9 Exploratory analysis of cervical cord cross-sectional area from MPRAGE brain acquisitions

Review of available data

All MPRAGE images were reviewed visually in sagittal, axial and coronal views using Mango image viewer (264) to establish

- 1) Presence or otherwise of required levels of spinal cord within available data, and
- 2) Visible degradation of data at that level (for instance by signal drop out or artefact) which was felt to preclude meaningful assessment of cord cross-sectional area.
 - a. For the latter estimate, because we aimed to establish the feasibility of these techniques, only data where meaningful assessment was felt to be impossible, was excluded.

T2 and FLAIR images were also reviewed visually to establish presence of demyelinating lesions at the levels studied. Study subjects in whom demyelinating lesions were present at the levels of study, were excluded from further analysis.

Image pre-processing

Volumetric MPRAGE images for each subject were reoriented and smoothed as described above (5.2.2)

The resulting MPRAGE images were viewed in Mango image viewer (264) and rotated, as necessary, in the x,y and z planes so that axial slices orthogonal to the cord could be extracted (118). The resulting images were then cropped at the level of the mid-pons rostrally, and inferior extent of the available image, caudally.

5.2.9.1.1 Cord cross-sectional area estimates: manual and automated

Cervical cord cross-sectional area was estimated at two separate levels:

- 2.5 cm below the inferior margin of the pons (termed here “P2.5” level (118)), and
- C2/3 level (termed here “C2/3”).

These levels were chosen as they were most reliable in terms of inter-rater reliability in one previous study (118) and C2/3 level is commonly used in studies of cervical cord cross-sectional area (CC CSA) (117).

Both manual and automated estimates were made for a subset of 15 subjects. For these purposes, 15 subjects were chosen at random using the function “sample” implemented within R (without resampling).

Manual estimation:

5.2.9.1.2 Level 2.5cm below pons (spinomedullary junction level)

Please see Figure 15 below for a summary of this process.

The caudal margin of the pons was identified visually, and a distance of 2.5cm below this level measured using Mango image viewer. This level was set as the rostral extent of an ROI for spinal cord cross-sectional area estimation.

The image was then viewed in the axial plane, and zoom of 300% applied in Mango image viewer. A manual “paint to fill” approach using the ROI estimation drawing tool (“ellipse” setting) in Mango image viewer was used to create a spinal cord cross-sectional area estimate. The same estimate was then made at a further 4 contiguous

slices, ie on 5 slices over a 5mm distance cranio-caudally. Mean cross-sectional area within this volume was then calculated (118) using Microsoft Excel .

5.2.9.1.3 *Level C 2/3 disc space*

The C2/3 disc space was identified using the sagittal MPRAGE images, and the middle of the anterior border of the disc space identified (118). This level was used as the middle slice of 5 contiguous 1mm slices over 5mm cranio-caudally, over which cross-sectional cord area was estimated manually using the procedure above.

Automated estimation of CC-CSA:

Cropped, reoriented MPRAGE images, as described above, were used to create a binary estimate of cervical cord using Spinal Cord Toolbox (266). Spinal cord segmentation used the ‘sct_propseg’ program within this toolbox, which has been validated in healthy controls, and two subjects with structural cord injury. This recently developed technique uses four steps (266):

- 1) Detection of the median plane of the image
- 2) Detection of potential spinal cord position, on multiple axial slices
 - a. This step uses an elliptical Hough transform, and may initially misidentify nearby structures of similar shape, such as carotid artery, as spinal cord
 - b. False positives are removed by discarding estimates which are not surrounded by spinal canal
- 3) Resulting chains of ellipses representing possible spinal cord boundaries are identified by neighbourhood analysis
- 4) A discriminant analysis determines correctness of the spinal cord border detection based on mean and standard deviation of the spinal cord radius, and cord:CSF signal contrast.

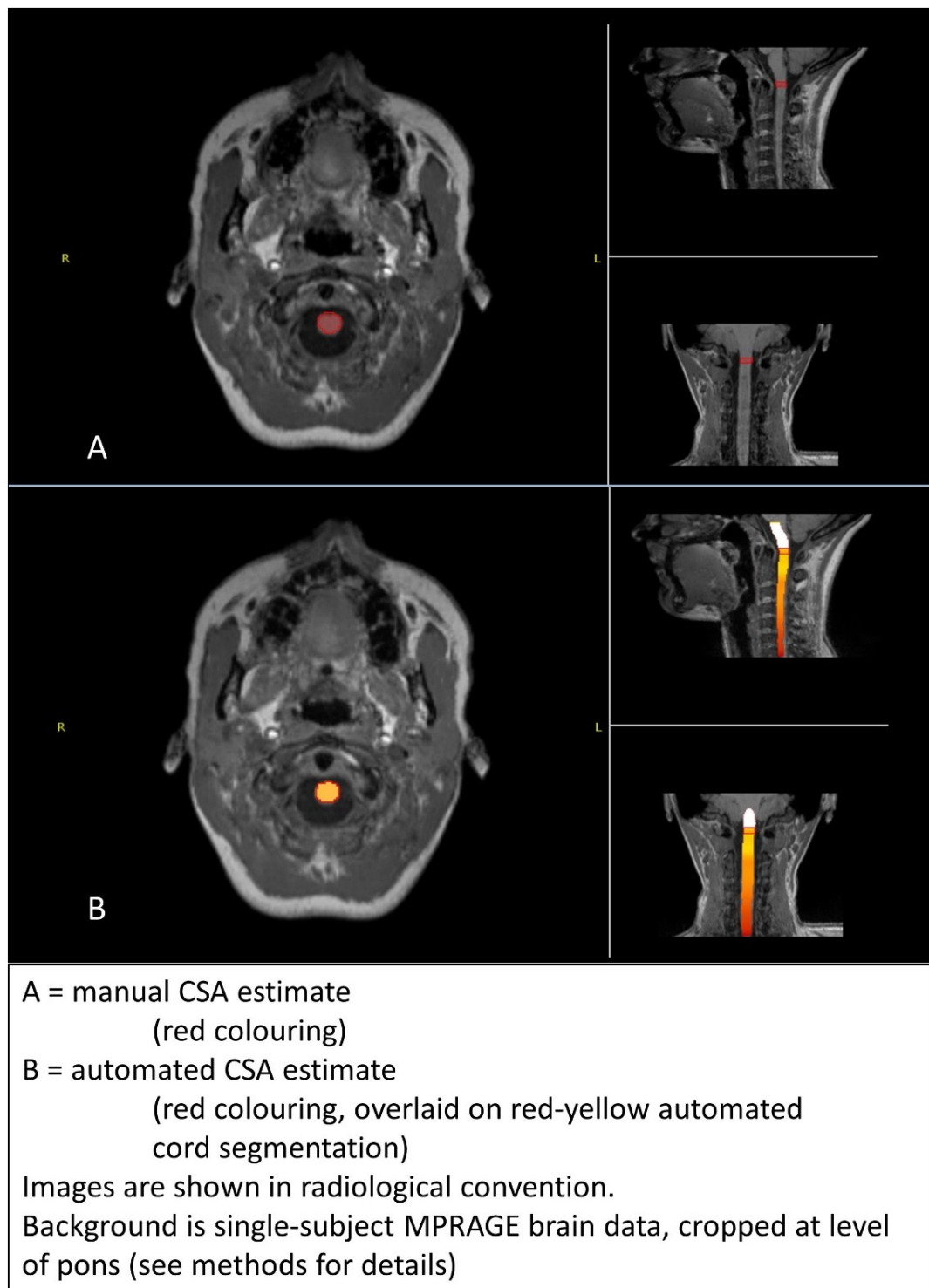
Registration to a standard space template, and/or tissue-type segmentation were not carried out. Binary cord segmentation estimates were reviewed visually for accuracy.

5.2.9.1.4 CC-CSA estimates without correction for image alignment

Automated CC-CSA estimates output by Spinal Cord Toolbox include a further correction for image alignment (266) . CC-CSA estimates output with this further correction might not be directly comparable to manual estimates.

In order to estimate cross-sectional area in a manner directly comparable to the manual segmentation described above, firstly manual ROIs were created by extending the manual cord cross-sectional area estimates described above, in the antero-posterior axis, beyond the extent of the spinal canal. The intersection of the spinal cord segmentation estimate generated by Spinal Cord Toolbox, and the manual spinal canal estimate described, was then calculated using `fslmaths`. This procedure was carried out to ensure that the CC-CSA estimates obtained from manual and automated methods were derived from identical levels of the cervical cord.

Figure 15: manual and automated estimates of cervical spinal cord cross-sectional area (spinomedullary junction level)



Comparison of automated and manual estimation

Automated and manual cord cross-sectional area estimates were then compared separately at the P2.5 and C2/3 level.

Data distribution was examined by histogram and QQ plot. Where data was not normally distributed, between-group differences were tested by Mann-Whitney U test (Wilcoxon rank sum test) and correlation by Spearman's Rho (137).

Cross-sectional cord area estimates were firstly compared visually (see Figure 1), then correlation examined using an exploratory scatterplot, and, if appropriate, Spearman's Rho. In order to further investigate mean difference between manual and automated estimates at each of these levels, and to look for associations between cord cross-sectional area and difference between the two described methods, a Bland-Altman plot was then constructed using the BlandAltman function available from the MethComp package in R (280).

Based on the above findings (see results), further analysis used automated estimates of cord cross-sectional area for the study group as a whole ($n=45$). Further automated estimates used CC-CSA estimates further adjusted for centreline orientation within Spinal Cord Toolbox compute_csa program.

Examining associations between cord cross-sectional area, imaging and clinical parameters

Exploratory correlations between cord cross-sectional area at both levels examined, radiological and clinical variables (namely EDSS and disease duration) were examined in a correlation matrix using Spearman's Rho. Associations with gender were separately examined using Wilcoxon's rank sum test and 95% CI of difference between groups. Because the optimal use of this data is not established, both 'raw' cross-sectional area, and CSA corrected for ICV, were examined.

Because correlations of cord cross-sectional area with EDSS and disease duration might be affected by pain severity in the pain group, but not the control group, these two groups were also examined separately in a further exploratory analysis.

Lastly cord cross-sectional area was compared between patients with and without neuropathic pain in this study. Throughout, significance levels were not corrected for multiple comparisons due to the exploratory nature of this substudy.

5.3 Results

5.3.1 Availability of data

Structural MRI brain imaging data was available for 45 of the 47 subjects who entered the study (29 of 31 subjects with neuropathic pain, 16 of 16 control subjects without neuropathic pain).

One subject in the pain group was unable to undergo MRI imaging because of claustrophobia, and the other because of a previously undisclosed contraindication to MRI imaging which became apparent at time of safety checks immediately prior to imaging.

5.3.2 Comparison of Intracranial Volume (ICV) estimation techniques

Visual comparison

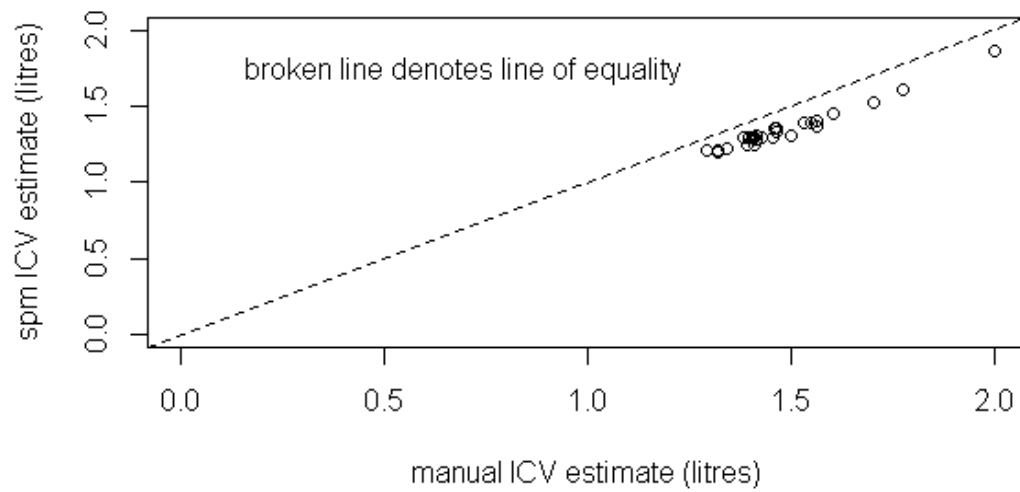
Semi-automated and automated estimates were compared in fslview for the 28 subjects included in the substudy comparing these techniques.

In all cases, results were comparable. SPM12 segmentations typically did not include venous sinuses within the intracranial volume, suggesting that ICV estimated by SPM might be systematically slightly lower than that estimated manually.

Correlation of automated and semi-automated ICV estimates

An exploratory scatterplot comparing automated and semi-automated approaches confirms that the ICV estimates output by the two approaches are highly correlated. The data are displayed alongside a hypothetical “line of equality”, where ICV estimates arising from the two approaches are identical. It can be seen that SPM ICV estimates tend to be lower than those arising from manual ICV estimation.

Figure 16: correlation of automated and semi-automated ICV estimates

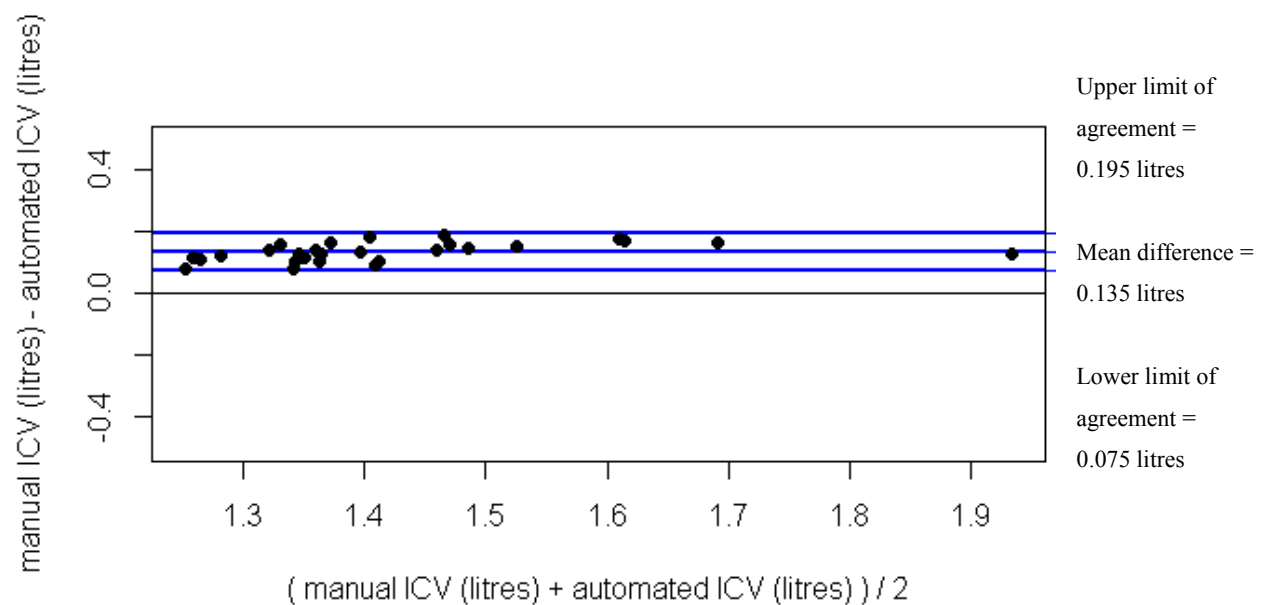


Spearman's Rho confirmed that the two estimates were highly correlated, as expected (Rho 0.94; $p = 8.6 \times 10^{-14}$)

Agreement between measurement techniques

Construction of a Bland-Altman plot confirms that automated ICV estimation tends to estimate ICV as 0.135 litres lower than manual estimates, on average (Figure 17). On visual inspection of the plot, no systematic association between mean ICV measured by the two techniques, and difference between the two techniques, is apparent.

Figure 17: Bland-Altman plot comparing manual and automated ICV estimates (n=28)



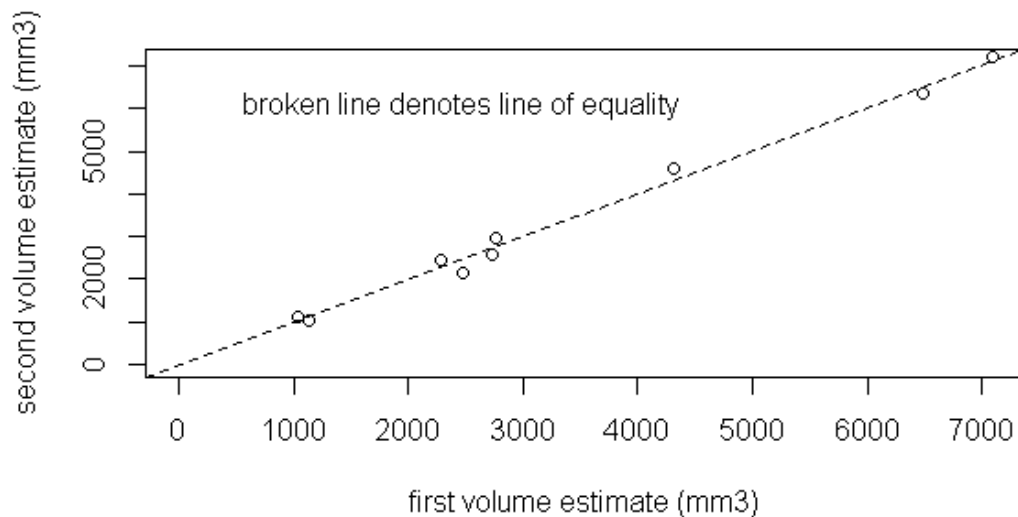
5.3.3 Intra-rater reliability of manual white matter lesion segmentation

Repeat white matter hyperintensity volume estimates were derived for 9 subjects. At the first analysis, mean lesion volume was 3369.3 mm³ (range 1033 to 7097). At the second analysis, mean lesion volume was 3387.9 mm³ (range 1046 to 7236). Median time elapsed between manual editing of lesion masks on the first and second occasions was 176 days (IQR 60 to 180 days, range 60 to 181 days).

Correlation

An exploratory scatterplot suggested that white matter hyperintensity volume estimates at first and second analysis were closely correlated. Results are plotted with a hypothetical “line of equality” along which first estimate would exactly match second estimate.

Figure 18: Scatterplot of first and second white matter hyperintensity manual lesion mask (n=9)

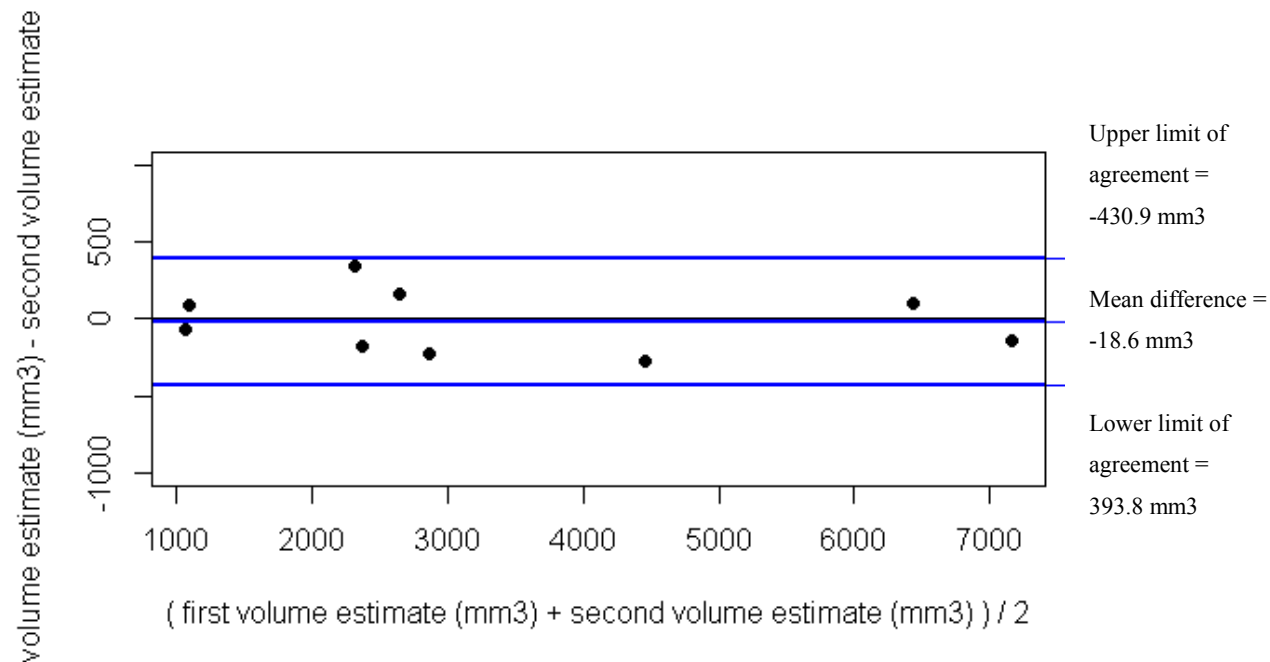


Intraclass correlation coefficient for this small sample was 1 (95% CI 0.98 to 1), suggesting high intra-rater reliability in this small sample.

Agreement between repeated measures

Bland Altman plot demonstrated that average lesion volume per subject on the second analysis was 18.56 mm³ lower than at first estimate, with 2.5% and 97.5% limits of agreement -430.9, and 393.8 mm³ respectively (Figure 19).

Figure 19: Bland-Altman plot comparing two manually edited lesion masks by single rater (n=9)



No relationship of subject lesion volume, and tendency to over- or under-estimate lesion volume in comparison with first estimation, was observed.

Intra-rater reliability of manual white matter lesion segmentation was judged to be acceptable.

5.3.4 Associations of the presence of pain: comparison of MS subjects with and without neuropathic pain

Volumes of overall tissue classes

No statistically significant difference was found between subjects with and without neuropathic pain, in Intracranial Volume, total Grey Matter Volume, or Brain Parenchymal Volume (Table 41).

Table 41: Comparison of tissue class volumes in pain and control groups

	Neuropathic Pain (n=29)	Control Group (n=16)	95% CI of difference	“p” value
Intracranial Volume (litres)	1.30 (1.28 to 1.40)	1.36 (1.34 to 1.44)	-0.12 to 0.04	0.23
Total Grey Matter Volume (% of ICV)	41.02 (38.45 to 43.20)	41.18 (39.41 to 42.31)	-2.10 to 1.65	0.86
Brain Parenchymal Fraction (% of ICV)	74.23 (71.73 to 76.23)	74.49 (72.49 to 75.68)	-1.68 to 2.39	0.97
ICV = Intracranial Volume				

Lesion volume and distribution

5.3.4.1.1 General description

T2 hyperintense lesions consistent with the known diagnosis of Relapsing Remitting Multiple Sclerosis were observed in all patients included in the study. Lesions were concentrated in the periventricular white matter, though distribution throughout the brain was variable, and included subcortical and brainstem locations.

5.3.4.1.2 T2 hyperintense lesion volume (whole brain, and brainstem)

Lesion volume (expressed as a percentage of ICV) was not significantly different between the pain and control groups. Lesion volume localised to the brainstem was significantly higher in the pain group than the control group ($p=0.0049$).

Table 42: overall lesion volume, whole brain and brainstem – comparison between pain group and control group.

	Control Group (% of ICV) (median, IQR) (n=16)	Neuropathic Pain (% of ICV) (median, IQR) (n=29)	“p” value
Total T2 visible lesion load	0.12 (0.06 to 0.42)	0.20 (0.12 to 0.42)	0.21
Brainstem T2 visible lesion load	0.00 (0.00 to 0.00052)	0.0021 (0.00025 to 0.0047)	0.0049

5.3.4.1.3 *Lesion topography: overall and by group*

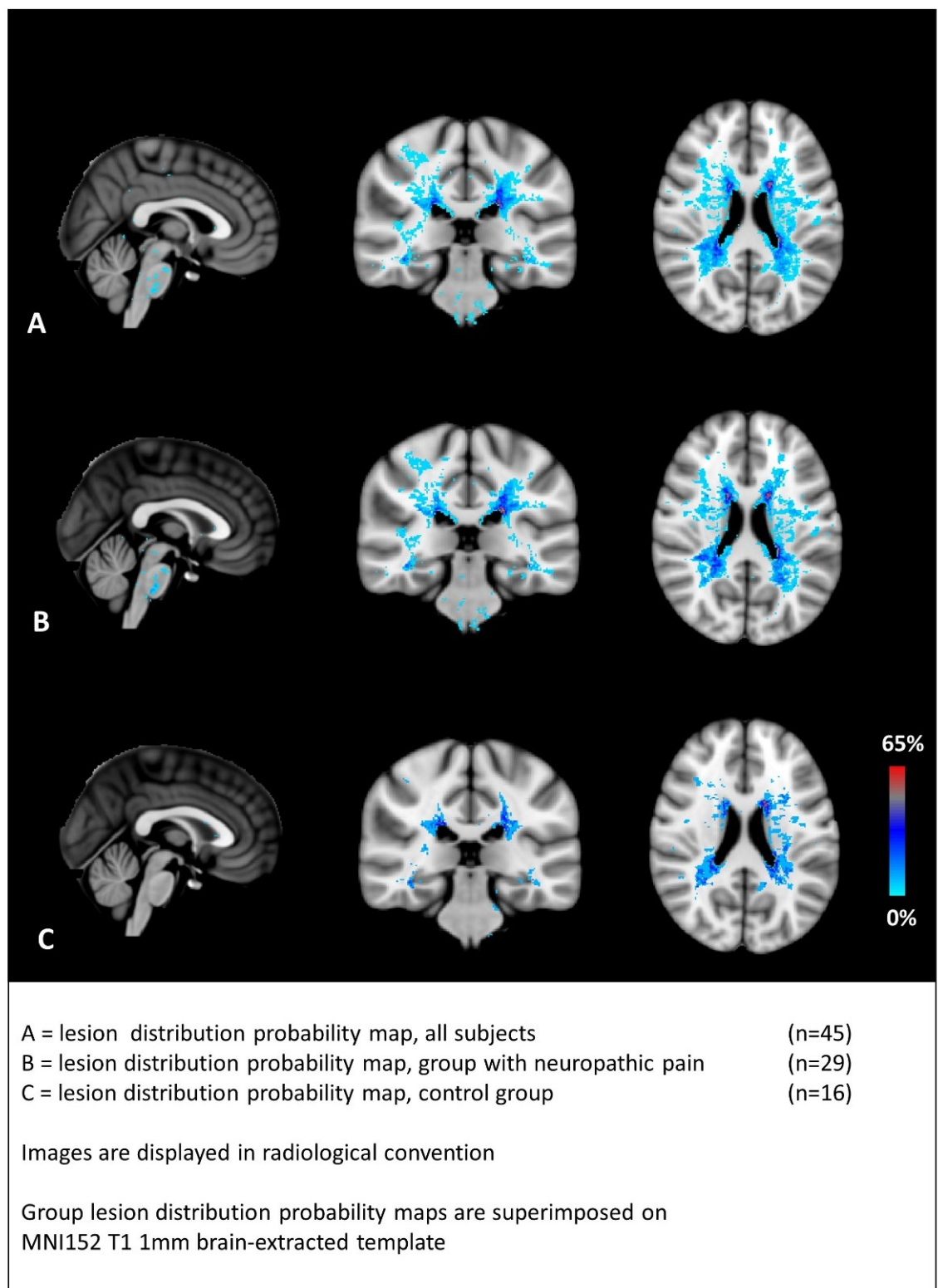
The highest probability of lesion location in the group as a whole was found at MNI coordinates $x = -16$, $y = -15$, $z = 27$, adjacent to the left lateral ventricle (percentage probability of lesion location 59.6%).

The highest probability of lesion location in the group with pain was found at MNI coordinates $x = 18$, $y = -17$, $z = 28$, adjacent to the right lateral ventricle (percentage probability of lesion location 69.0%).

The highest probability of lesion location in the control group was found at MNI coordinates $x = -17$, $y = -13$, $z = 27$, adjacent to the left lateral ventricle (percentage probability of lesion location 62.5%).

See below (Figure 20) for lesion probability maps throughout the study group ($n = 45$) as a whole, as well as pain group ($n = 29$) and control group ($n = 16$) separately.

Figure 20: Lesion distribution probability maps: Whole group, pain group and control group (n=45 total)



5.3.4.1.4 *Lesion topography: comparison by subtraction maps*

For the contrast between pain group and control group, lesion distribution probability “difference maps” are shown in Figure 21. This figure shows the distributions where lesion location is more likely in the pain group (without correction for multiple comparisons) and where lesion location is more likely in the control group (without correction for multiple comparisons) as estimated by simple subtraction.

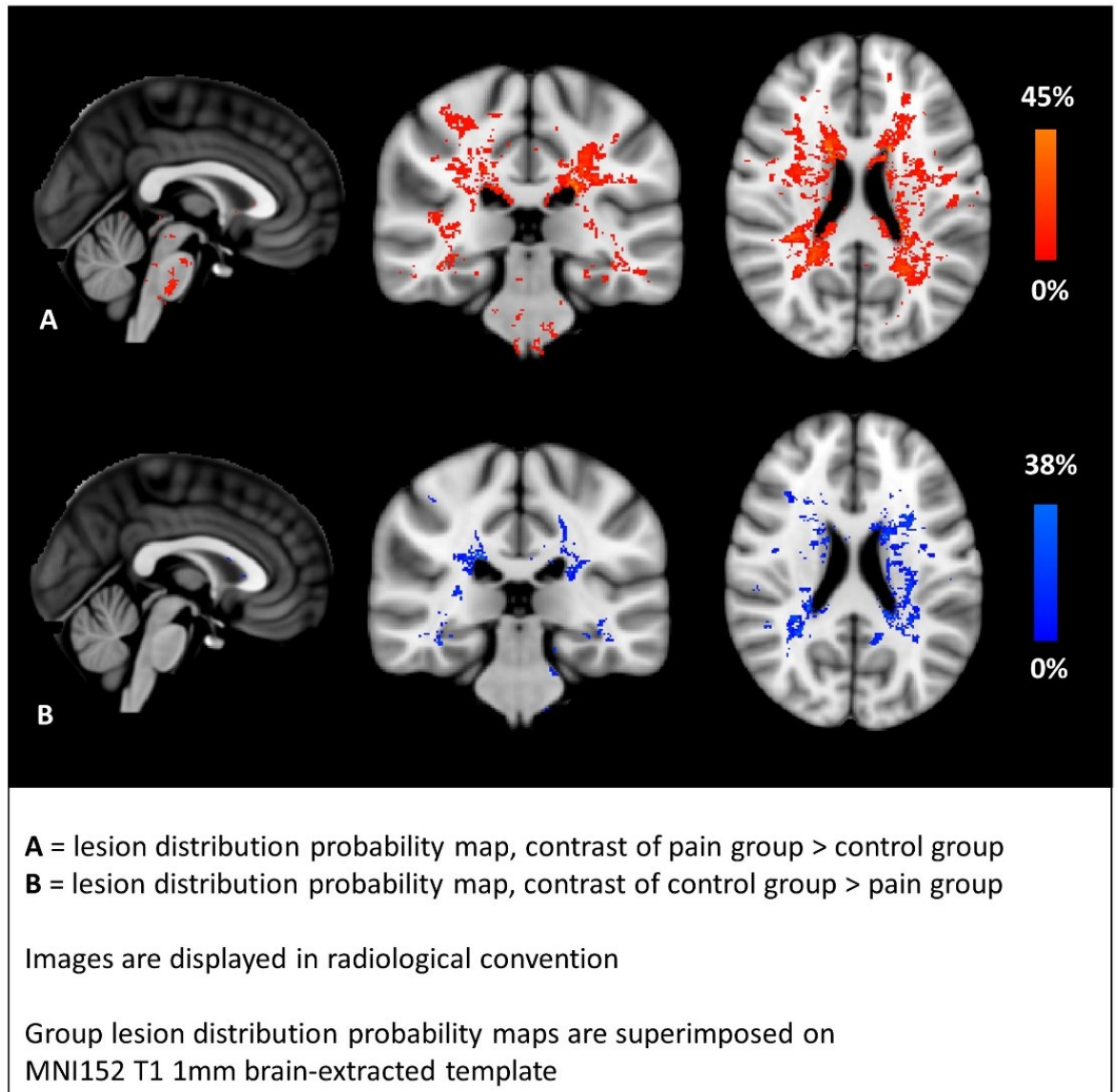
The highest likelihood of lesion location in the pain group relative to the control group was at MNI coordinates $x=-17$, $y=-22$, $z=27$, adjacent to the left lateral ventricle (probability = 44.9%).

The highest likelihood of lesion location in the control group relative to the pain group was at MNI coordinates $x=-27$, $y=-51$, $z=15$, also adjacent to left lateral ventricle (probability = 35.6%).

On visual comparison of the two difference maps, no clear difference was observed in supratentorial white matter hyperintensity distribution. The volume of voxels where lesion location was more likely in the pain group than the control group appeared higher than the volume of voxels where lesion location was more likely in the control group. Lesions located in the brainstem appeared more likely in the pain group than the control group on visual comparison.

Please see Figure 21.

Figure 21: Difference in lesion probability maps, pain and control group, by groupwise subtraction



5.3.4.1.5 *Lesion topography (whole brain) at 5% significance level, corrected for multiple comparisons*

No statistically significant difference in lesion topography was found between pain and control groups, at the 5% significance level, and corrected for multiple comparisons in space.

5.3.4.1.6 *Lesion topography (whole brain) at 0.1% significance level, uncorrected for multiple comparisons*

In an exploratory analysis using a less stringent threshold of $p < 0.001$, uncorrected for multiple comparisons across the whole brain, only a single voxel exceeded significance thresholds. This voxel was located at MNI coordinates $x=22, y=-76, z=7$ (adjacent to the occipital horn of the right lateral ventricle). This finding is of doubtful clinical significance and should be interpreted with caution.

5.3.4.1.7 *Lesion topography (brainstem region of interest) at 5% significance level, corrected for multiple comparisons*

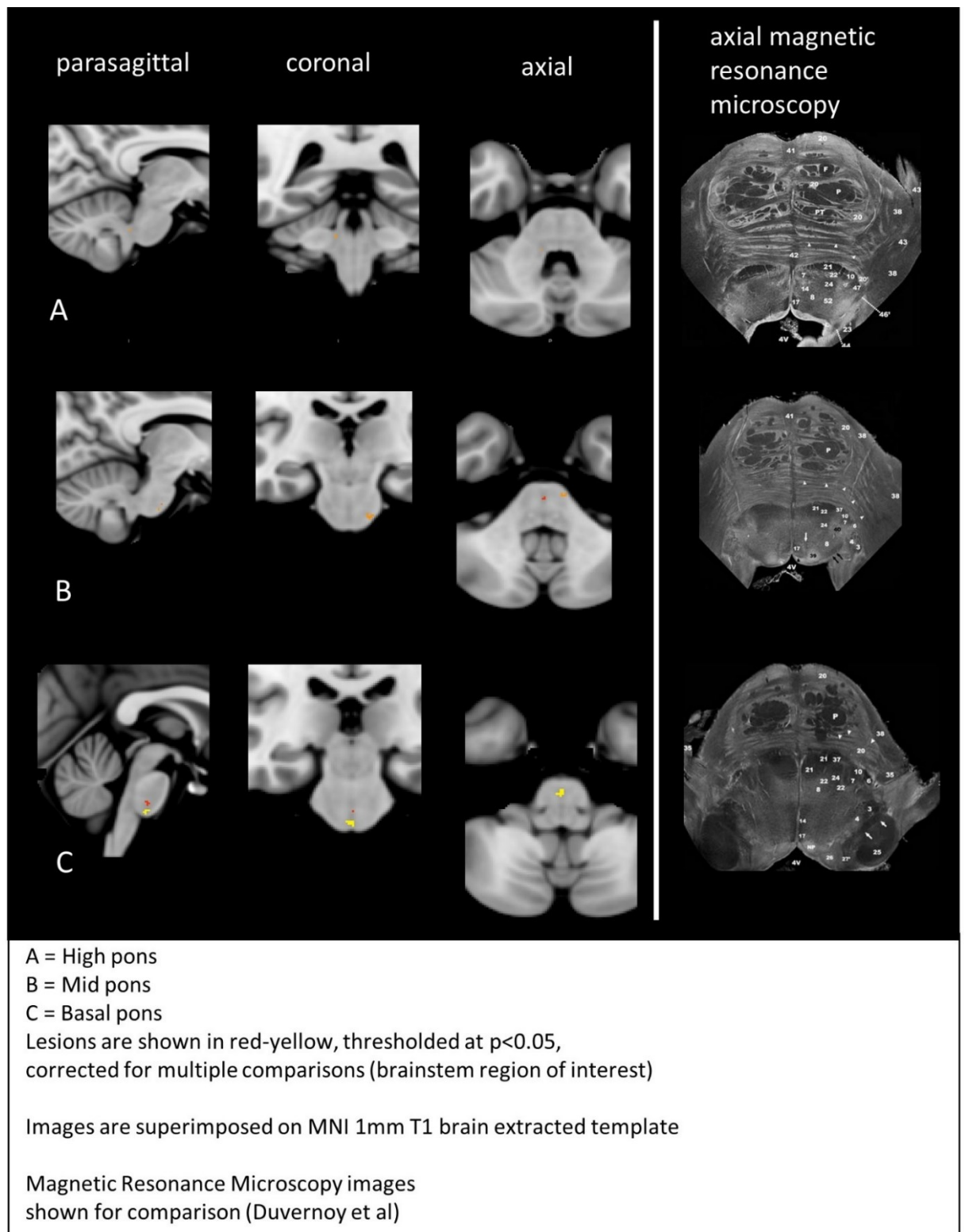
In a brainstem-restricted region of interest analysis, at 5% significance level and corrected for multiple comparisons in space, lesions were more likely to occur in the pain group at the following locations (Table 43; Figure 22).

Table 43: anatomical location of T2 hyperintense lesions more likely in pain group than controls

MNI coordinates			Region	anatomical correlate on inspection of axial magnetic resonance microscopy images (Duvernoy atlas (273))
x	y	z		
10	-39	-34	Right cerebellar peduncle	cerebellar peduncle
-10	-24	-40	Lateral mid-pons	Arcuate nuclei of pons
0	-27	-45	Basal pons	Arcuate nuclei of pons/pontine decussation

No lesion location was found to be more likely to occur in the control group.

Figure 22: Brainstem lesion topography - locations of T2 hyperintense lesions more likely in pain group than controls



Voxel Based Morphometry

5.3.4.1.8 Whole grey matter analysis, 5% significance level corrected for multiple comparisons

At the 5% significance level, with correction for multiple comparisons, no difference in grey matter volume was found between subjects with and without neuropathic pain.

5.3.4.1.9 Whole grey matter analysis, 0.1% significance level uncorrected for multiple comparisons.

In an exploratory whole-grey-matter analysis where $p < 0.001$, uncorrected for multiple comparisons in space, differences in grey matter volumes were seen in the following locations (Table 44, Figure 23, Figure 24).

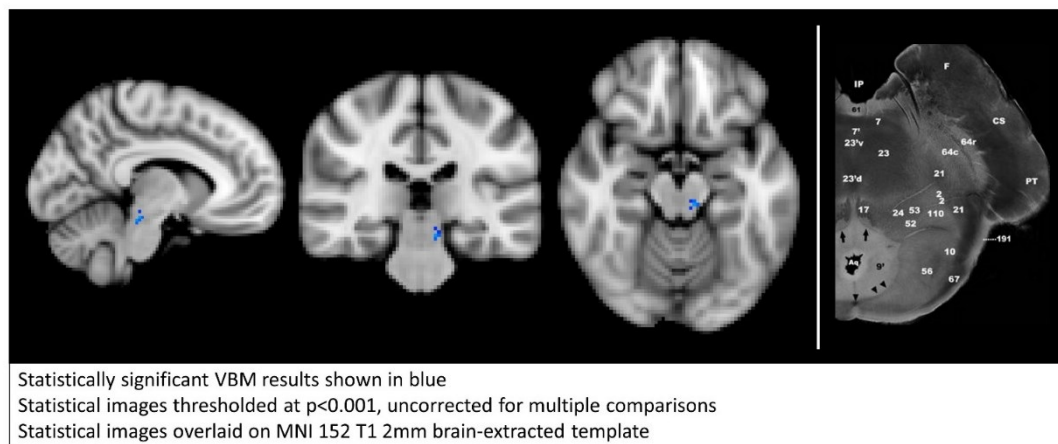
Table 44: Localisation of VBM result; exploratory threshold $p < 0.001$ uncorrected

	MNI coordinates			Anatomical localisation (116, 272)
	x	y	z	
Volume greater in control group	-6	-38	28	Mid-posterior cingulate, bilateral but centred on left
	24	-16	-32	Right parahippocampal gyrus
	50	-74	28	Right occipital lobe
Volume greater in pain group	-8	-32	-16	Left midbrain, trigeminothalamic nucleus

Figure 23: *Voxel based morphometry - grey matter volume greater in control group than pain group; exploratory threshold $p < 0.001$ uncorrected*



Figure 24: Voxel based morphometry: grey matter volume greater in pain group than control group; exploratory threshold $p < 0.001$ uncorrected



5.3.4.1.10 Grey matter analysis, restricted to mask of descending pain modulatory system, 5% significance level corrected for multiple comparisons

No difference in grey matter volume was found at the 5% significance level.

5.3.4.1.11 Whole grey matter analysis, 5% significance level uncorrected for multiple comparisons (for the purpose of comparison with previously published data (214))

Widespread differences were found between the pain and control groups at this significance threshold, which could be regarded as statistically relaxed. Conclusions drawn from these data should be limited. These are listed below, with particular emphasis on structures known to be relevant to central processing of pain.

5.3.4.1.11.1 Structures where grey matter volume less in those with neuropathic pain, than those without:

- Cerebellum: right cerebellar cortex and vermis
- Orbitofrontal cortex (bilaterally)
- Bilateral temporal lobes (mesial and lateral) including hippocampi but not amygdalae
- Bilateral occipital lobes

- Bilateral frontal pole
- Bilateral insular cortex (anterior and posterior)
- Bilateral posterior cingulate, bilateral precuneous

5.3.4.1.11.2 Structures where grey matter volume greater in pain group than control group:

- Bilateral cerebellar hemispheres posteriorly
- Bilateral pons and midbrain including PAG
- Bilateral amygdalae
- Bilateral thalamus
- Bilateral anterior cingulate
- Bilateral postcentral gyrus
- Right superior parietal lobule

5.3.5 Correlates of pain severity

Volumes of overall tissue classes

No statistically significant association of Intracranial Volume, Grey Matter Volume or Brain Parenchymal Fraction with pain severity was found (Table 45).

Table 45: Correlations of tissue class volumes with pain severity

	Spearman's Rho	"p" value
Intracranial Volume (litres)	-0.21	0.26
Total Grey Matter Volume (% of ICV)	0.12	0.53
Brain Parenchymal Volume (% of ICV)	0.23	0.24

Lesion volume and distribution

5.3.5.1.1 T2 hyperintense lesion volume (whole brain)

Whole-brain T2 hyperintense lesion volume was not significantly correlated with pain severity (Spearman's Rho -0.20, $p=0.29$)

5.3.5.1.2 T2 hyperintense lesion volume (brainstem only)

Brainstem-restricted T2 hyperintense lesion volume was not significantly correlated with pain severity (Spearman's Rho -0.36, $p=0.056$)

5.3.5.1.3 Lesion topography (whole brain) at 5% significance level, corrected for multiple comparisons.

Lesion topography was not correlated with pain severity at the 5% significance level, corrected for multiple comparisons across the whole brain using Randomise. No specific lesion location was positively or negatively associated with the severity of pain.

5.3.5.1.4 Lesion topography (whole brain) at 0.001% significance level, uncorrected for multiple comparisons across space

Lesion topography was not correlated with pain severity at the $p<0.001$ level, uncorrected for multiple comparisons in space. No specific lesion location was positively or negatively associated with the severity of pain.

5.3.5.1.5 *Lesion topography (brainstem region of interest), 5% significance level, corrected for multiple comparisons*

Lesion topography was not correlated with pain severity at the 5% significance level, corrected for multiple comparisons in space across the brainstem.

Voxel Based Morphometry

5.3.5.1.6 Whole grey matter analysis, 5% significance level corrected for multiple comparisons

No association of regional grey matter volume with pain severity was found

5.3.5.1.7 Whole grey matter analysis, exploratory 0.1% significance level, uncorrected for multiple comparisons

In this exploratory analysis, no regional grey matter volume change was significantly associated with pain severity, in either the positive or negative direction. Some regional grey matter volumes closely approached the statistical threshold applied, however are not reported here.

5.3.5.1.8 Grey matter analysis, restricted to mask of descending pain modulatory system, 5% significance level

No association of regional grey matter volume with pain severity within the described mask was identified.

5.3.6 Grey matter correlates of pain duration

Positive correlation of grey matter volume with pain duration

5.3.6.1.1 *Whole grey matter analysis, 5% significance level corrected for multiple comparisons*

No local grey matter volumes were positively correlated with pain duration at this significance level

5.3.6.1.2 *Grey matter analysis restricted to DPMS mask, 5% significance level corrected for multiple comparisons*

No local grey matter volumes were positively correlated with pain duration at this significance level

5.3.6.1.3 *Whole grey matter analysis, 0.1% significance level not corrected for multiple comparisons*

No local grey matter volumes were positively correlated with pain duration at this exploratory significance level

Negative correlation of grey matter volumes with pain duration

5.3.6.1.4 *Whole grey matter analysis, 5% significance level corrected for multiple comparisons*

No local grey matter volumes were positively correlated with pain duration at this significance level

5.3.6.1.5 *Grey matter analysis restricted to DPMS mask, 5% significance level corrected for multiple comparisons*

No local grey matter volumes were positively correlated with pain duration at this significance level

5.3.6.1.6 *Whole grey matter analysis, 0.1% significance level not corrected for multiple comparisons*

Lower grey matter volumes in the left anterior cingulate (x51,y59,z56) were negatively correlated with pain duration at this exploratory significance level.

5.3.7 Exploratory analysis of cross-sectional cord area

Availability of data

Structural brain MRI MPRAGE data was available for 45 subjects. All brain MRI data included the P2.5 level. 44 of 45 (97.8%) included the C2-3 level.

Quality of data

All images allowed an estimation of cord cross-sectional area. CC-CSA could be estimated in 45 subjects at P2.5 level, and 44 at C2/3 level. No images were excluded on the basis of poor quality. No subjects were excluded because of presence of demyelinating cord lesions at the levels studied.

Manual and automated cross-sectional area at P2.5 (spinomedullary junction)

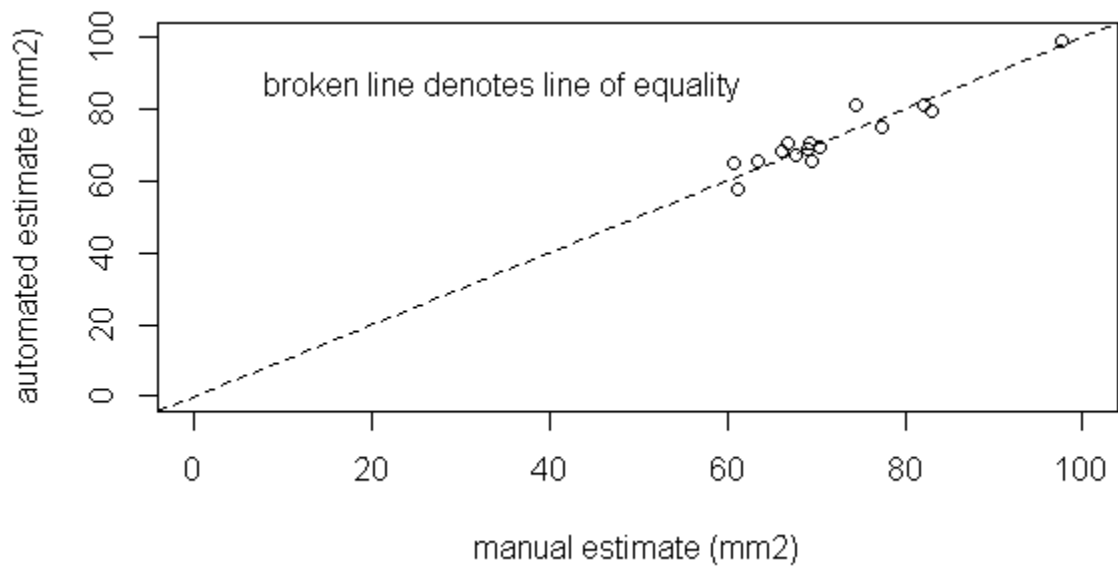
5.3.7.1.1 *Performance of automated segmentation – visual inspection*

The techniques described were successful in generating estimates of cord cross-sectional area in all cases. No misidentification of adjacent structures (such as the carotid artery) occurred. Estimates arising from manual and automated techniques were visually comparable in all cases.

5.3.7.1.2 *Correlation*

An exploratory scatterplot confirmed close correlation across the range of available data in this small sample (Figure 25), presented with line of equality for the hypothetical case where automated estimate = manual estimate, for comparison). Mean cervical cord cross sectional area derived by automated and manual methods at the P2.5 level were highly correlated (Spearman's Rho 0.87, $p=2.2 \times 10^{-5}$).

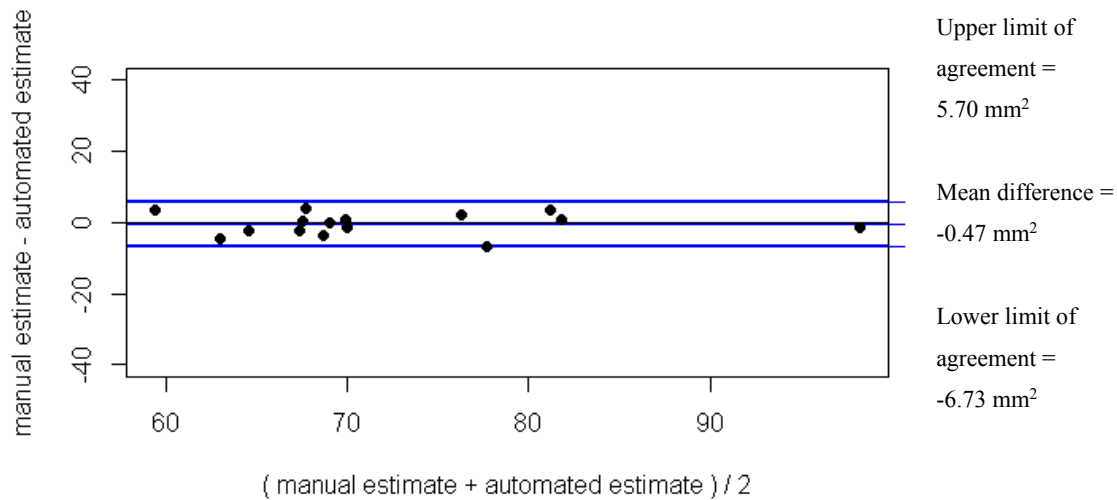
Figure 25: scatterplot of manual and automated CSA estimates at P2.5 (spinomedullary junction level) (n=15)



5.3.7.1.3 Bland-Altman plot

A Bland-Altman plot confirmed close agreement of the two methods across the range of data in this small sample, with a mean difference of 0.47 mm² (automated estimate tending to be larger, on average), and 2.5% and 97.5% limits of agreement -6.73 and 5.80 mm² respectively. On visual inspection of the plot, no association between cord cross-sectional area and difference between estimation methods was apparent.

Figure 26: Bland Altman plot comparing manual and automated CSA estimates at P2.5 (spinomedullary junction level) (n=15)



Manual and automated cross-sectional area at C2/3 level

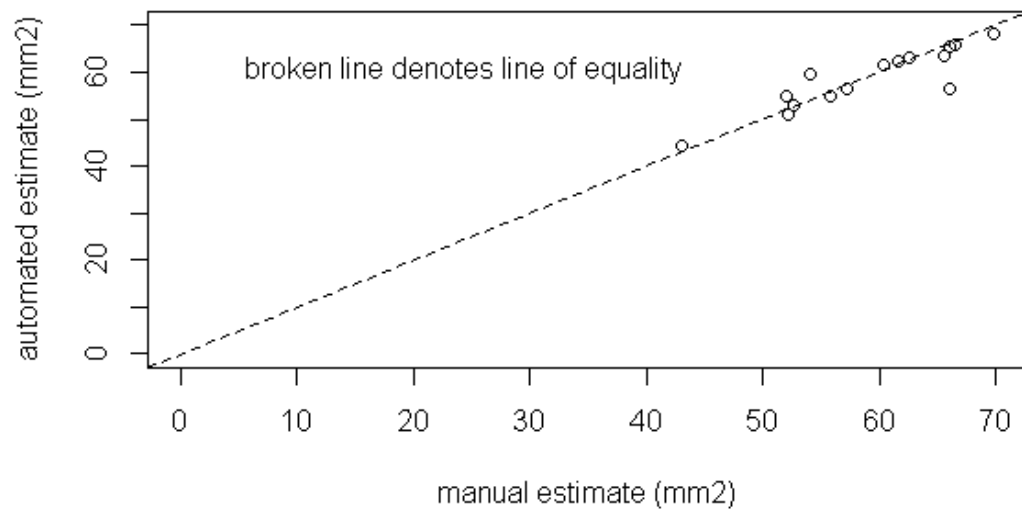
5.3.7.1.4 Visual inspection

The techniques described were successful in generating estimates of cord cross-sectional area in all cases at the C2/3 level. No misidentification of adjacent structures (such as the carotid artery) occurred. Estimates arising from manual and automated techniques were visually comparable in all cases.

5.3.7.1.5 Correlation

An exploratory scatterplot confirmed close correlation across the range of available data in this small sample (Figure 4). Data are shown with the hypothetical line of equality, along which manual and automated estimates would be identical.

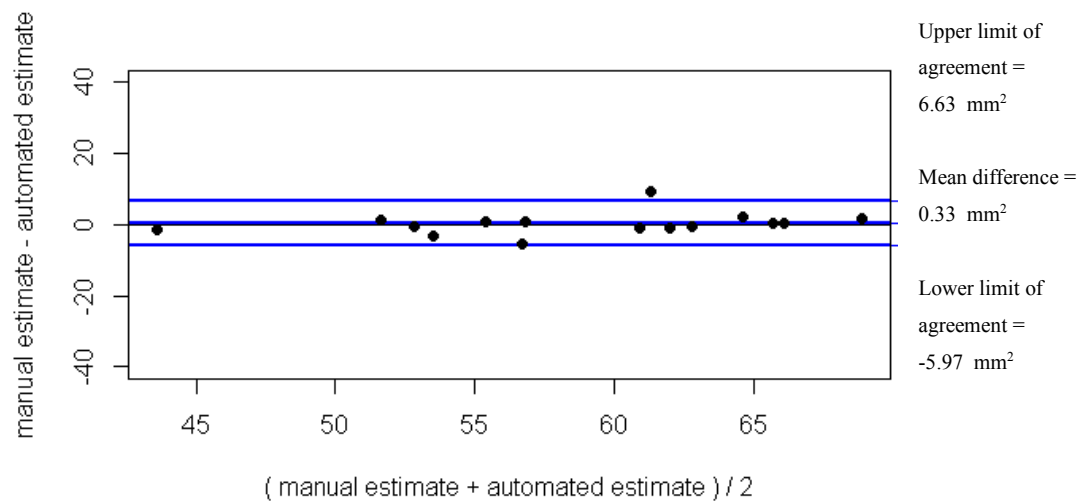
Mean cervical cord cross sectional area derived by automated and manual methods at the C2/3 level were highly correlated (Spearman's Rho 0.90, $p=4.4 \times 10^{-6}$).

Figure 27: Scatterplot comparing manual and automated CSA estimates (C2/3 level)

5.3.7.1.6 Bland Altman plot

A Bland-Altman plot confirmed close agreement of the two methods across the range of data in this small sample, with a mean difference of 0.33 mm² (manual estimate tending to be larger, on average), and 2.5% and 97.5% limits of agreement -5.97 and 6.63 mm² respectively. On visual inspection of the plot, no association between cord cross-sectional area and difference between estimation methods was apparent (Figure 28).

Figure 28: Bland Altman plot comparing manual and automated techniques for CSA estimation (C2/3 level)



Use of automated estimates for further analysis

Because manual and automated estimates of CC-CSA were highly correlated, and Bland-Altman plots were judged to be satisfactory in a subgroup of 15 randomly selected patients, automated estimation was used for further analysis in the entire subject group (n=45).

Correlation between CC-CSA estimates and ICV.

CC-CSA at P2.5 and C2/3 levels were positively correlated both with and without correction for ICV (Spearman's Rho 0.53, uncorrected $p < 0.001$, and 0.56, uncorrected $p < 0.001$ respectively).

The correlation between CC-CSA at P2.5 level and ICV was stronger than at C2/3 level (Spearman's Rho 0.32, uncorrected $p = 0.03$; and 0.13, uncorrected $p = 0.39$ respectively).

These data are further explored in Table 46.

Table 46: Spearman correlations between automated cord cross-sectional area estimates, and intracranial volume (n=45)

	CSA, P2.5	CSA divided by ICV, P2.5	CSA, C2/3	CSA divided by ICV, C2/3	ICV
CSA, P2.5 (mm ²)	-				
CSA divided by ICV, P2.5	0.80 (p<0.001)	-			
CSA, C2/3 (mm ²)	0.53 (p<0.001)	0.50 (p<0.001)	-		
CSA divided by ICV, C2/3	0.28 (p=0.07)	0.56 (p<0.001)	0.79 (p<0.001)	-	
ICV	0.32 (p=0.03)	-0.23 (p=0.13)	0.13 (p=0.39)	-0.43 (p=0.003)	-
CSA= mean cervical cord cross-sectional area measured across 5 contiguous 1mm slices C2/3= C2/C3 disc level P2.5 = 2.5 cm below caudal margin of pons. ICV=intracranial volume 'p' values are not corrected for multiple comparisons in this exploratory analysis					

Relationships between CC-CSA, ICV and clinical variables

5.3.7.1.7 All subjects

Examining the study group as a whole (n=45, subjects with and without pain), EDSS was weakly correlated with CC-CSA at both P2.5 and C2/3 level (with or without correction for ICV) (see Table 47).

Disease duration was moderately/highly correlated with CC-CSA at the P2.5 level, when expressed as a fraction of ICV. Other correlations, including with CC-CSA at the C2/3 level, were weak (Table 47).

EDSS and disease duration were only weakly correlated (Spearman's Rho 0.12, $p=0.43$) in the group as a whole.

CC-CSA tended to be higher in males than females at both the P2.5 and C2/3 levels, before correction for ICV, though differences did not attain statistical significance (Table 48).

Table 47: Correlations between cord cross-sectional area estimates, intracranial volume, disability score and disease duration (n=45)

	CSA, P2.5 (mm ²)	CSA/ ICV, P2.5	CSA, C2/3 (mm ²)	CSA/ ICV, C2/3	ICV	EDSS	Disease Duration (years)
CSA, P2.5 (mm ²)	-						
CSA/ ICV, P2.5	0.80 (p<0.001)	-					
CSA, C2/3 (mm ²)	0.53 (p<0.001)	0.50 (p<0.001)	-				
CSA/ ICV, C2/3	0.28 (p=0.07)	0.56 (p<0.001)	0.79 (p<0.001)	-			
ICV	0.32 (p=0.03)	-0.23 (p=0.13)	0.13 (p=0.39)	-0.43 (p=0.003)	-		
EDSS	0.04 (p=0.80)	0.02 (p=0.89)	-0.02 (p=0.91)	-0.08 (p=0.61)	0.10 (p=0.52)	-	
Disease Duration (years)	0.07 (p=0.65)	0.44 (p=0.12)	-0.10 (p=0.53)	0.01 (p=0.93)	-0.14 P=0.36	0.12 (p=0.43)	-
<p>CSA= mean cervical cord cross-sectional area measured across 5 contiguous 1mm slices at specified level</p> <p>C2/3= C2/C3 disc level</p> <p>P2.5 = 2.5 cm below caudal margin of pons.</p> <p>ICV=intracranial volume</p> <p>Significance thresholds are not corrected for multiple comparisons in this exploratory analysis</p>							

Table 48: variations in cervical cord cross-sectional area measures by gender (n=45)

	Gender: female	Gender: male	95% CI of difference	'p' value
CSA, P2.5 (mm ²) (median, IQR)	55.61 (52.44 to 61.35)	62.40 (56.79 to 74.40)	-0.15 to 18.53	0.058
CSA/ ICV, P2.5 (median, IQR)	41.86 (38.73 to 47.00)	45.61 (39.82 to 47.13)	-2.87 to 5.31	0.665
CSA, C2/3 (mm ²) (median, IQR)	47.02 (43.36 to 49.66)	51.60 (41.77 to 55.72)	-4.56 to 10.09	0.44
CSA/ ICV, C2/3 (median, IQR)	34.78 (31.93 to 39.95)	32.74 (30.02 to 34.10)	-6.85 to 1.02	0.194
<p>CSA= mean cervical cord cross-sectional area measured across 5 contiguous 1mm slices at specified level ICV = Intracranial Volume</p> <p>C2/3= C2/C3 disc level P2.5 = 2.5 cm below caudal margin of pons. ICV=intracranial volume</p> <p>Significance thresholds are not corrected for multiple comparisons in this exploratory analysis</p>				

Comparison of cord cross-sectional area in participants with and without pain

CC-CSA at P2.5 level was not significantly different in subjects with and without neuropathic pain, whether expressed as a fraction of ICV or not.

Median CC-CSA at C2/3 level after correction for ICV was 32.49 mm² in subjects without pain as compared to 35.71 mm² in those with pain. This difference did reach statistical significance at the 5% significance level (uncorrected for multiple comparisons) however is reported only for purposes of data exploration.

Table 49: Comparison of CC-CSA in study participants with and without neuropathic limb pain

	Participants with pain (n=29)	Participants without pain (n=16)	95% Confidence Interval of Difference	“p” value
Cross-sectional area at C2/3 level, as fraction of ICV (median, IQR)	35.71 (33.34 to 38.37)	32.49 (29.88 to 33.67)	0.58 to 6.88	0.02
Cross-sectional cord area at C2/3 level (mm ² , median, IQR)	48.31 (45.28 to 51.85)	45.63 (41.65 to 45.58)	-1.23 to 6.81	0.13
Cross-sectional cord area at P2.5 level, as fraction of ICV (median, IQR)	41.91 (39.10 to 47.35)	42.41 (38.73 to 45.29)	-2.43 to 4.43	0.50
Cross-sectional cord area at P2.5 level (mm ² , median, IQR)	57.05 (52.09 to 58.73)	55.61 (54.03 to 62.17)	-4.39 to 4.58	0.93
Mean CSA= mean cervical cord cross-sectional area measured across 5 contiguous 1mm slices C2/3= C2/C3 disc level P2.5 = 2.5 cm below caudal margin of pons. ICV=intracranial volume				

5.4 Discussion

The current data explores possible relationships between the previously-described clinical and neuropsychological associations of the presence and severity of neuropathic limb pain in RRMS, and MRI measures of brain structure. Some trends to association were identified, for instance using the exploratory statistical threshold of $p < 0.001$ uncorrected for multiple comparisons (which has been reported previously in the neuroimaging literature (80, 288)

Please see the Thesis Discussion (page 271) for a full description and discussion of findings.

At an exploratory statistical threshold, the pain group manifests lower grey matter volumes in the posterior cingulate and right parahippocampal gyrus, as well as higher volumes in the left midbrain trigeminothalamic nucleus area, adjacent to the periaqueductal grey matter. Increasing duration of pain is also negatively associated with grey matter volume in the anterior cingulate cortex. These findings are consistent with findings in previous studies of pain syndromes outside MS.

Associations of increasing pain duration with decreased grey matter volume may suggest a causative link between the experience of pain and grey matter volumes, though further longitudinal studies would be needed to explore this hypothesis.

T2 hyperintense MS lesions are found to be more common in specific brainstem locations in the pain group, when using a region of interest analysis. These findings, which should be viewed with caution, may be relevant to a disconnection syndrome, reflecting the complex interaction of white matter and grey matter abnormalities in MS (78, 85). Regional grey matter volume differences between the groups in pre-specified structures usually recognised to be key to the descending modulation of pain, were not found. The potential relevance of posterior cingulate and parahippocampal gyrus in pain, memory and attentional roles, all of which are highly relevant to the current study are discussed.

In addition, an early exploration of cervical cord cross-sectional area measurements extracted from the current data using open source software has been included. This

analysis may suggest that further exploration of the reproducibility, reliability and utility of this technique is warranted.

Chapter 6 **Functional connectivity of the descending pain modulatory system examined using resting state functional MRI**

6.1 Introduction

Chapters Four and Five of this thesis describe assessments of a range of variables relevant to the descending modulation of pain, in people with relapsing remitting multiple sclerosis, with and without neuropathic limb pain. Specifically, findings related to the descending pain modulatory system (DPMS) are emphasised.

This chapter aims to further examine the functional integrity of this system, using resting state functional MRI (fMRI), in participants with and without neuropathic limb pain. Structures which are key to the endogenous modulation of pain are emphasised, specifically the rostral Anterior Cingulate Cortex (rACC), Dorsolateral Prefrontal Cortex (DLPFC) and Periaqueductal Grey matter (PAG).

Please see the Thesis Introduction (page 1) for a detailed discussion of background, aims and hypotheses, and for a review of the functional neuroanatomy of the DPMS (page 9).

6.2 Methods

6.2.1 Estimation of sample size

Overall sample size estimation has been based on sample size estimation for the functional MRI component of the study. I viewed fMRI outcomes of the study as the primary outcomes of interest in the study overall.

Sample size/power calculations would ideally be based on existing functional MRI studies of people with relapsing remitting MS, comparing groups with and without pain, and using a design identical to (or at least comparable to) the current study. Specific tools are available to employ such pilot data in sample size calculations for fMRI studies (for instance the fmripower website (75)). Unfortunately, applicable pilot data was not available for the current study.

At the time of design of this study, no published fMRI studies examined differences between people with RRMS, with and without neuropathic pain (166). During recruitment to this study, one functional MRI study comparing subjects with RRMS, with and without neuropathic pain was published (214).

Existing studies are briefly reviewed in order to give context with regards to sample sizes.

Existing studies: pain in pwMS examined using resting state fMRI

One study from Professor Tracey's group (214) used fMRI to assess resting state functional connectivity networks in people with MS who experienced pain, compared to those who did not. This study used a mixed sample of people with relapsing remitting, secondary progressive and primary progressive MS. Resting state fMRI was used, and analysis carried out according to "dual regression" methodology to examine and compare resting state networks (289). This study used a total sample size of 23 (group sizes 12 and 11). This study was able to discern a difference in resting connectivity of the default mode network between the groups with and without pain.

No published studies in pain related to MS, however, have used a seed based analysis in common with the current study.

Functional imaging associations of neuropathic limb pain in MS

Existing studies: pain in healthy volunteers or subjects with other health conditions examined using resting state fMRI

Published fMRI studies of other pain syndromes are typically carried out in patients with structurally normal brains, so that results are not directly comparable to the current study, in which MS lesions could be expected to alter the blood:brain barrier (even in normal-appearing tissue). Any such effects, in turn, could influence BOLD signal (290). Thus data from conditions other than MS may be used for general comparison however cannot be used for sample size estimation.

Mainero and colleagues compared PAG connectivity in 17 subjects with migraine, and 17 age and gender-matched controls (103). Eippert and colleagues compared connectivity of PAG and other structures in 24 healthy controls receiving a sham intervention, with 24 healthy controls not receiving this intervention (110). Yu and colleagues compared functional connectivity of PAG in 18 subjects with chronic low back pain, and 18 healthy controls (115).

A priori attempts to estimate ideal sample size in fMRI case-control studies (in the absence of applicable pilot data) have included statistical analyses by Professor Friston (291, 292). Estimation of sample size was also discussed with my supervisory team, and others including Dr Yazhuo Kong (FMRIB). Professor Friston's analyses suggest that a group size of approximately 16 in an fMRI study provides an optimal balance of sensitivity to medium/large effect sizes, while also minimising sensitivity to trivial effects. (292).

Estimation for current study

Based on the above work, it was not possible to calculate a precise required sample size on the basis of existing studies. In addition, methods for calculating sample size for resting state fMRI studies are not well developed.

Given the case:control design of the study, a minimum group size of 16 was estimated to be sufficient. In order to maximise sensitivity to associations of pain severity, within the pain group, recruitment to the pain group was maximised within time and practical constraints associated with the study.

Functional imaging associations of neuropathic limb pain in MS

6.2.2 Acquisition

Scanner

A single Siemens Verio scanner (3 Tesla, 12 channel head coil) was used as detailed in Chapter Five. No scanner upgrades were carried out during the study.

Field maps

Phase and magnitude field maps were acquired using standard Siemens acquisitions, with a total acquisition time of 1 minute 5 seconds.

Resting state functional data

Echo-planar images (EPI) were acquired with the following characteristics:

Repetition time (TR) = 3000ms, Echo Time (TE) = 30ms, flip angle = 90 degrees, 46 slices, field of view (FOV) = 192mm, 3mm slice thickness, interleaved acquisition, acquisition time = 5 minutes 23 seconds.

Clinical data acquired in scanner

Following field map acquisition, and immediately prior to resting state acquisitions, subjects were given the following questions:

- “could you tell me, on a scale of zero to ten, how much pain you are experiencing just now, due to the MS?”

and

- “could you tell me, on a scale of zero to ten, how unpleasant that pain is?”

These responses were recorded as estimates of pain severity and unpleasantness at time of imaging.

Instructions to participants

Participants were then instructed to lie still, with their eyes open and to think of nothing in particular during the resting state fMRI acquisition, without falling asleep.

Functional imaging associations of neuropathic limb pain in MS

6.2.3 Preprocessing of resting state data

Preparation of fieldmap data

Two magnitude images were output by the scanner in a single folder. These were separated using a custom-written shell script written by Stephen Giles (University of Edinburgh).

Siemens fieldmap data was prepared using `fsl_prepare_fieldmap` in the command line.

In order to optimise brain extraction of the magnitude field maps (and specifically to maximise brain coverage while avoiding inclusion of non-brain tissue) standard brain extraction (`f0.7`) was supplemented with erosion of the mask by one voxel using `fslmaths`. Brain extraction including non-inclusion of skull data was checked manually.

Preprocessing

Preprocessing of resting state data was carried out in FEAT version 6.00, implemented in FSL (105). The Graphical User Interface (GUI) was used.

The following steps were followed:

The first three volumes were deleted to allow equilibration of signal (115).

A high pass filter cutoff of 90 seconds was applied

Head motion was calculated using MCFLIRT implemented in FSL.

Slice timing correction was applied (using a Fourier-space time-series phase-shifting)

Spatial smoothing with a full width half maximum (FWHM) kernel of 5mm (115) (approximately double voxel size).

Independent Components Analysis (ICA) implemented in FSL's MELODIC (105) suite was used to investigate the possible presence of unexpected artefacts (103).

Grand-mean intensity normalisation of the entire 4dimensional data set by a single multiplicative factor

Functional imaging associations of neuropathic limb pain in MS

Registration

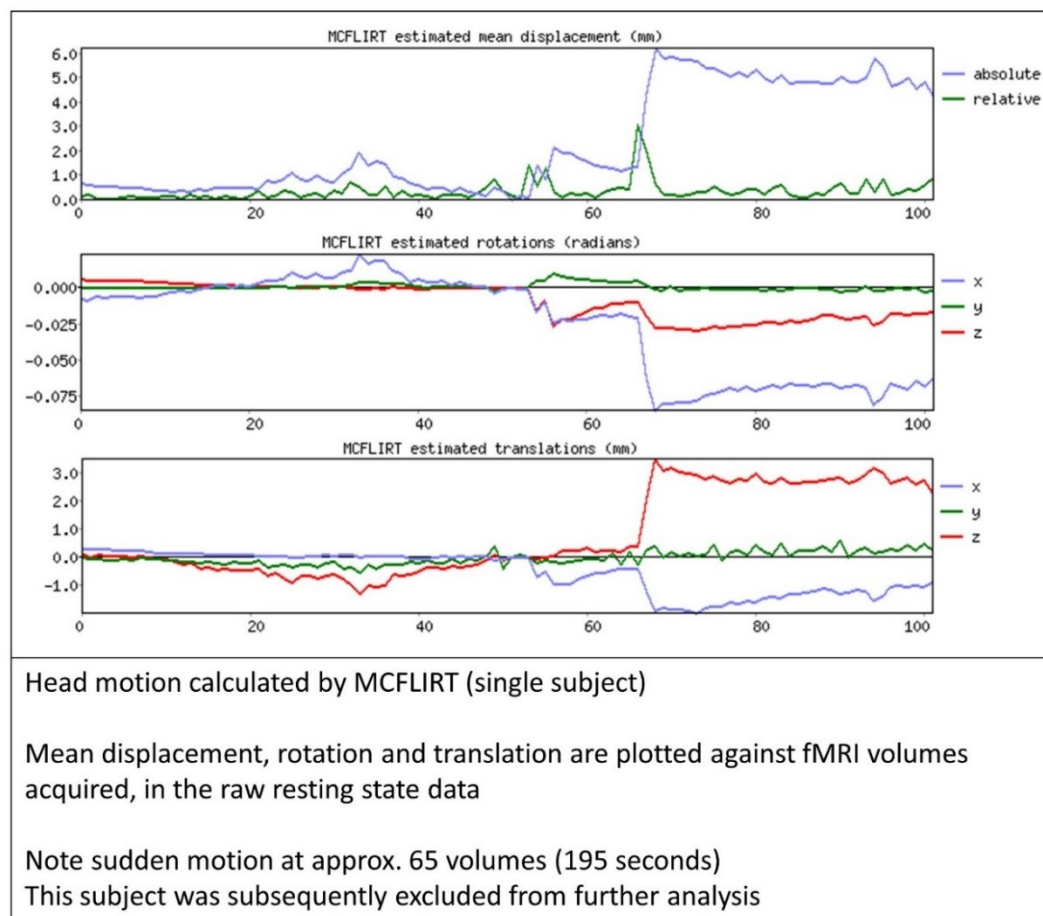
Each subject's resting state images were registered to their own brain-extracted MPAGE data using a linear registration in FSL's FLIRT, including a boundary based registration (BBR) implemented in FEAT (293). This tool is designed for registration of EPI to structural images, and uses white-matter:grey-matter boundaries to optimise registration. Fieldmap unwarping was included in the registration pipeline, as implemented in FEAT, in order to minimise any effect of B0 inhomogeneity (105).

Each subject's resting state data was then registered to the MNI152 2mm brain-extracted template using a non-linear transformation in FSL's FNIRT (274), with 12 degrees of freedom.

Approach to potential sources of artefact and noise

6.2.3.1.1 *Head movement: gradual rotation/translation*

Rotational and translational movements of the head were calculated using MCFLIRT and included as covariates of no interest in the GLM model. For clarity and for reference, Figure 29 (below) shows an example of mixed gradual and sudden head motion.

Figure 29: Example of sudden head motion during resting state fMRI acquisition

6.2.3.1.2 Exclusion of subjects with significant head movement

All subjects in whom absolute head movement was equal to or greater than one millimetre during the resting state acquisition were excluded from subsequent analysis.

For each subject, absolute head motion was recorded, and compared between the pain and control groups using a Wilcoxon Rank Sum test.

6.2.3.1.3 Identification of structured noise using independent components analysis at individual subject level

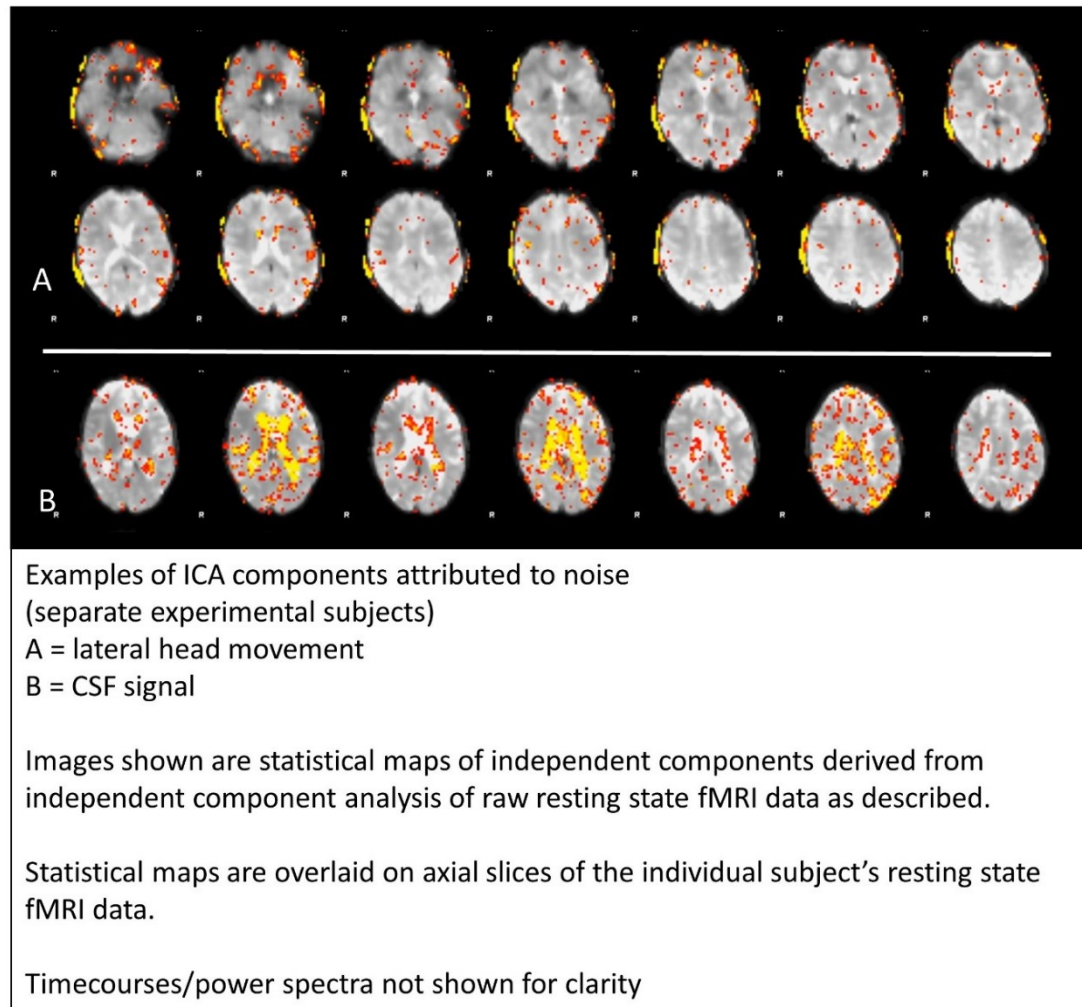
As detailed above, MELODIC was used to identify structured noise in the resting state data.

Functional imaging associations of neuropathic limb pain in MS

During preprocessing in FEAT, each subject's data were decomposed into components on the basis of their spatial and temporal characteristics, without an explicit time series or number of components being specified.

These components were reviewed in a web browser with particular attention to their spatial distribution and timecourse, and compared to published descriptions of noise sources in resting state analysis (75, 104). Components thought to represent subject head movement, CSF signal, white matter signal or other signals of no interest (including cardiorespiratory noise and scanner artefact) were noted (see Figure 30), and regressed out using `fsl_regfilt`. A relatively conservative ("non-aggressive") approach was deliberately used, and where there was any doubt as to the possible neuronal origin of an identified component, it was not removed. This approach echoes others described in the literature (100). Please see Figure 30 for examples of structured noise identified using this approach.

Figure 30: Examples of structured noise sources identified in resting state data using ICA



6.2.3.1.4 Regression of white matter and CSF signal at subject level

White matter and CSF masks were hand-drawn in standard space (using the MNI 2mm brain-extracted template), over the corona radiata and lateral ventricles respectively, using FSLview.

White matter and CSF masks were transformed to each individual subject's resting state functional data using a nonlinear registration implemented in FSL's FNIRT. Specifically, the inverse of the transformation from each subject's resting state functional data to standard space (including boundary based registration and fieldmap unwarping) implemented in FEAT as described above (see Para 6.2.3) was used.

Functional imaging associations of neuropathic limb pain in MS

Registration of white matter and CSF masks to each individual subject's resting state functional data was checked visually using FSLview.

Timecourses were extracted using *fslmeants*, and the timecourses for WM and CSF masks combined into a single file. These timecourses were subsequently employed as nuisance covariates ("covariates of no interest") in the subject-level resting state data analysis in FEAT.

6.2.4 Creation of region of interest masks

Three region of interest masks were used for fMRI analysis. These structures were chosen as core components of the descending pain modulatory system. A seed mask was created centred on the rostral anterior cingulate cortex. Two further region of interest masks were centred on the periaqueductal grey matter and bilateral dorsolateral prefrontal cortex.

For creation of each of these masks, previously published descriptions were considered in conjunction with data from the current study where appropriate. Please see Figure 31 for a summary.

Rostral Anterior Cingulate Cortex (rACC) seed mask

6.2.4.1.1 *Delineation of extent of rACC*

As a guide for delineation of the boundaries of rACC, a mask created by Vishvarani Wanigasekera (FMRIB) was used. This bilateral mask of the rACC was created by first thresholding the Oxford-Harvard anterior cingulate cortex probabilistic mask (116) at 50% probability, and then setting the caudal boundary as defined by Vogt and colleagues (294).

6.2.4.1.2 *Previous literature*

In order to further delineate the volume of interest for rACC seed placement, a previous analysis of resting state connectivity of the anterior cingulate was used (Margulies and colleagues) (295). This work used systematically placed multiple resting state seeds to delineate connectivity of the anterior cingulate. The most rostrally placed bilateral seeds (described as "superior 7" or "s7") were identified as

Functional imaging associations of neuropathic limb pain in MS

of particular interest because of functional connectivity with frontal cortex structures implicated in the cognitive modulation of pain, as well as temporal lobe structures. These seeds were located at MNI coordinates $x \pm 5$, $y=47$, $z=11$. Other authors (110) have used 6mm spheres centred around each subject's maximal PAG:ACC connectivity.

6.2.4.1.3 *Mask for this study*

Based on the above, a 6mm spherical mask was set in the midline centred on MNI coordinates $x=0$, $y=42$, $z=8$. Total volume of this mask was 984 mm^3 . Please see Figure 31

Periaqueductal grey matter (PAG) region of interest mask

Seed placement was drawn from a previous publication by Eippert and colleagues, which investigated the functional connectivity of the periaqueductal grey matter in relation to the descending pain modulatory system (110). A binarised spherical PAG mask of 6mm radius was created, centred on MNI coordinates $x=0$ $y=-32$ $z=-10$. Total mask volume was 984mm^3 . Please see Figure 31.

Bilateral dorsolateral prefrontal cortex (DLPFC) region of interest mask

6.2.4.1.4 *Delineation of outer extent of DLPFC*

Masks of the dorsolateral prefrontal cortex were kindly supplied by Dr Katja Wiech, FMRIB centre, University of Oxford. These were created as previously described (65). Briefly, masks were hand-drawn on T1 images based on Brodmann areas 8, 9, 46, 9/46 (approximately corresponding to the superior and middle frontal gyrus) (65). These masks were used to delineate the boundaries of DLPFC for creation of a region of interest mask.

6.2.4.1.5 *Previously published DLPFC seed masks*

Zubieta and colleagues (296) report the localisation of DLPFC involvement in placebo response as maximal at $x=-36$ $y=13$ $z=39$. Eippert and colleagues used the same coordinates (based on Zubieta's publication and ignoring laterality) as the kernel for a spherical mask (110).

6.2.4.1.6 *Study data*

Maximum mean rACC connectivity to the DLPFC was calculated (within the widest extent of the DLPFC as defined by Dr Wiech's mask), using all 42 subjects (with and without pain) in a higher-level analysis implemented in FEAT (FLAME 1) in FSL. Maximal mean functional connectivity of rostral ACC to DLPFC was identified at MNI coordinates $x=46$, $y=20$, $z=44$ on the right, lateral to the coordinates identified by Zubieta and colleagues (296) and within the spherical ROI specified by Eippert and colleagues (110). This location was identified as middle frontal gyrus bilaterally (using published estimates packaged with FSL (116)).

6.2.4.1.7 *Creation of seed mask for current study*

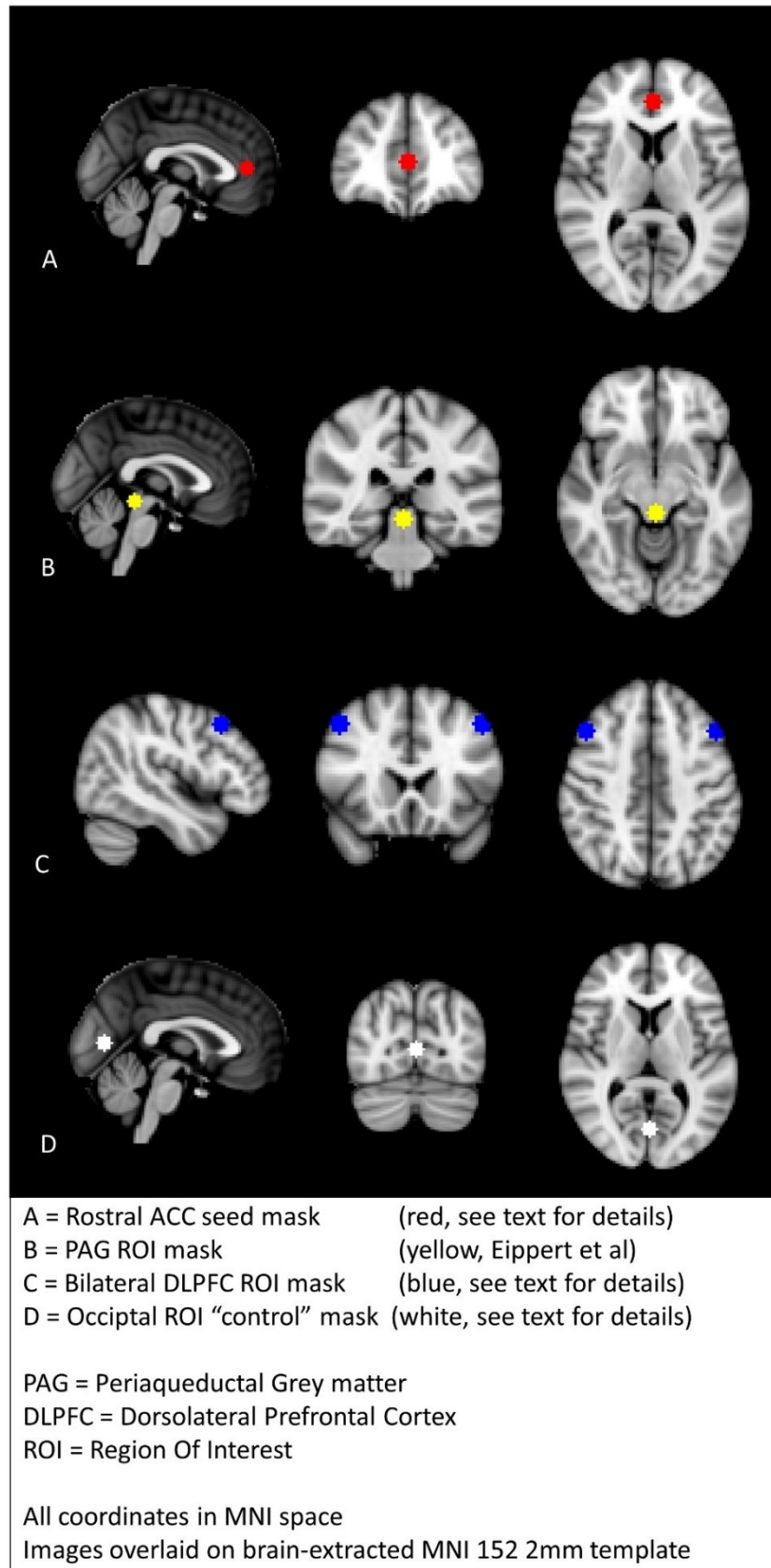
A binarised spherical mask of 6mm radius was created centred on these MNI coordinates. An identical mask was then created on the left, mirrored across the x axis. These masks were combined to create a single bilateral DLPFC mask consisting of two spheres of 6mm radius. The location of the bilateral DLPFC mask was confirmed as within the larger anatomical DLPFC mask defined by Wiech and colleagues (65). Mask boundaries extending beyond grey matter were removed using grey matter tissue probability estimates packaged with FSL, thresholding at intensity of 50. Total mask volume was 1808 mm³. Please see Figure 31.

Occipital cortex region of interest “control” mask

In order to test specificity of any findings to the DPMS, a further 6mm radius spherical binary region of interest was created around the MNI coordinates $x=0$, $y=-74$, $z=8$ (mid-occipital cortex) using the methods described above.

The occipital cortex is not thought to be implicated in the descending modulation of pain. The mask was deliberately placed in the midline ($x=0$), similarly to the rACC seed described, and was of the same volume as the rACC and PAG seeds. This mask therefore was intended for use in examining the specificity of any findings to the DPMS. The mask was placed as anteriorly as possible, while still avoiding any structures known to be involved in the DPMS, and also avoiding CSF voids other than the interhemispheric fissure.

Figure 31: Seed mask and Region of Interest masks used in this chapter



Functional imaging associations of neuropathic limb pain in MS

Registration of rACC seed mask to resting state functional data

For each subject, binarised masks (as described above) for the rostral ACC were registered to each subject's ICA-processed resting state data, using the reverse of the linear and nonlinear registration procedures already established using FEAT, which included fieldmap unwarping. Successful registration was checked visually for every subject.

6.2.5 Analysis of fMRI data

Extraction of timecourses at subject level

For each subject, the timecourse of the rACC mask (6.2.4) was extracted from resting state data which had been subject to ICA decomposition and regression of nuisance components as described above. In order to extract the timecourse, `fslmeants` was used ("fsl mean timeseries").

The resultant timecourse was used in group analyses (see below) as the independent variable in a general linear model examining correlation with timecourses of all other voxels in the brain. For each subject, and as described, CSF timecourse, white matter timecourse, and six head motion parameters were included as covariates of no interest.

Group level analyses

Group analyses were carried out using a mixed effects model implemented with FMRIB's Local Analysis of Mixed Effects (FLAME) version 1 implemented in FEAT.

6.2.5.1.1 Analyses within groups

6.2.5.1.1.1 Group means

For within-group analyses a one-sample analysis for the pain and control groups separately was used. A Z threshold of 2.3 and cluster forming $p \leq 0.05$ were used to create z-score statistical maps of rACC seed functional connectivity.

6.2.5.1.1.2 Correlations of rACC functional connectivity with T2 hyperintense lesion volume

Because the influence of T2 lesion volume on rACC functional connectivity is unknown, and because previous studies have described patterns of increased or decreased connectivity, or both, tests were carried out for any such correlation in the current data.

T2 hyperintense lesion volumes were expressed for each subject as a fraction of intracranial volume as described previously.

In order to calculate correlations of rACC seed functional connectivity with lesion volume, data from all 42 subjects (26 with pain, 16 controls) was used, and T2 hyperintense lesion volume was specified as a covariate of interest in a general linear model implemented in FEAT. Positive and negative correlations of rACC seed functional connectivity with lesion volume were calculated.

6.2.5.1.1.3 Correlations of rACC functional connectivity with pain severity

In order to calculate correlations of rACC seed functional connectivity with pain severity at the time of imaging, all 26 subjects with pain were included in a general linear model implemented in FEAT. Pain severity at the time of imaging was included as a covariate of interest. Positive and negative correlations of rACC seed functional connectivity with pain severity were calculated.

6.2.5.1.2 *Between-group analyses*

6.2.5.1.2.1 Whole brain

A between-group comparison between the pain and control groups was carried out using an unpaired two-sample t-test design, across the whole brain. A Z threshold of 2.3, and cluster forming $p \leq 0.05$ were used.

6.2.5.1.2.2 Regions of interest (PAG and DLPFC)

A between-group comparison within specified regions of interest (PAG and DLPFC as described in 6.2.4) was then calculated using nonparametric permutation testing implemented with Randomise, packaged in FSL. Threshold Free Cluster Extent was

Functional imaging associations of neuropathic limb pain in MS

calculated using 5000 permutations (as described in Chapter Five for VBM analysis, page 188) including correction for multiple comparisons in space.

6.2.5.1.3 *Incorporation of T2 lesion volume in models*

In order to examine any effects of T2 hyperintense lesion volume on findings in the above analyses, the above analyses were repeated with correction for T2 hyperintense lesion volume. Analyses were carried out as specified above, with inclusion of T2 lesion volume as an additional covariate of no interest.

6.3 Results

6.3.1 Clinical data

6.3.1.1.1 *Head movement and exclusion of subjects*

Two subjects in whom head movement of equal to, or over one millimetre over the course of the recording were excluded (2 subjects, absolute movement 1.33 millimetres and 2.28 millimetres). Both of these subjects were in the neuropathic pain group.

Subsequent analyses included 26 subjects in the neuropathic pain group, and 16 in the control group.

6.3.1.1.2 *Head movement at group level*

In the remaining 42 subjects, head movement in the pain group and control group was not significantly different (pain group: $n=26$ median 0.24mm, IQR 0.16 to 0.30, control group $n=16$ median 0.20mm, IQR 0.16 to 0.26, $p=0.42$).

6.3.1.1.3 *Pain severity and unpleasantness scores in the neuropathic pain group*

In the neuropathic pain group ($n=26$), median pain severity immediately prior to resting state fMRI acquisition (range 0-10) was 2.25 (IQR 1.00 to 7.00).

Pain unpleasantness in the same group (scored 0-10) was median 3.00 (IQR 1.00 to 7.00).

Ratings of pain and pain unpleasantness were highly correlated (Spearman's Rho 0.93, $p=1.8 \times 10^{-12}$). Because of this collinearity, functional connectivity correlates of pain unpleasantness ratings were not separately calculated.

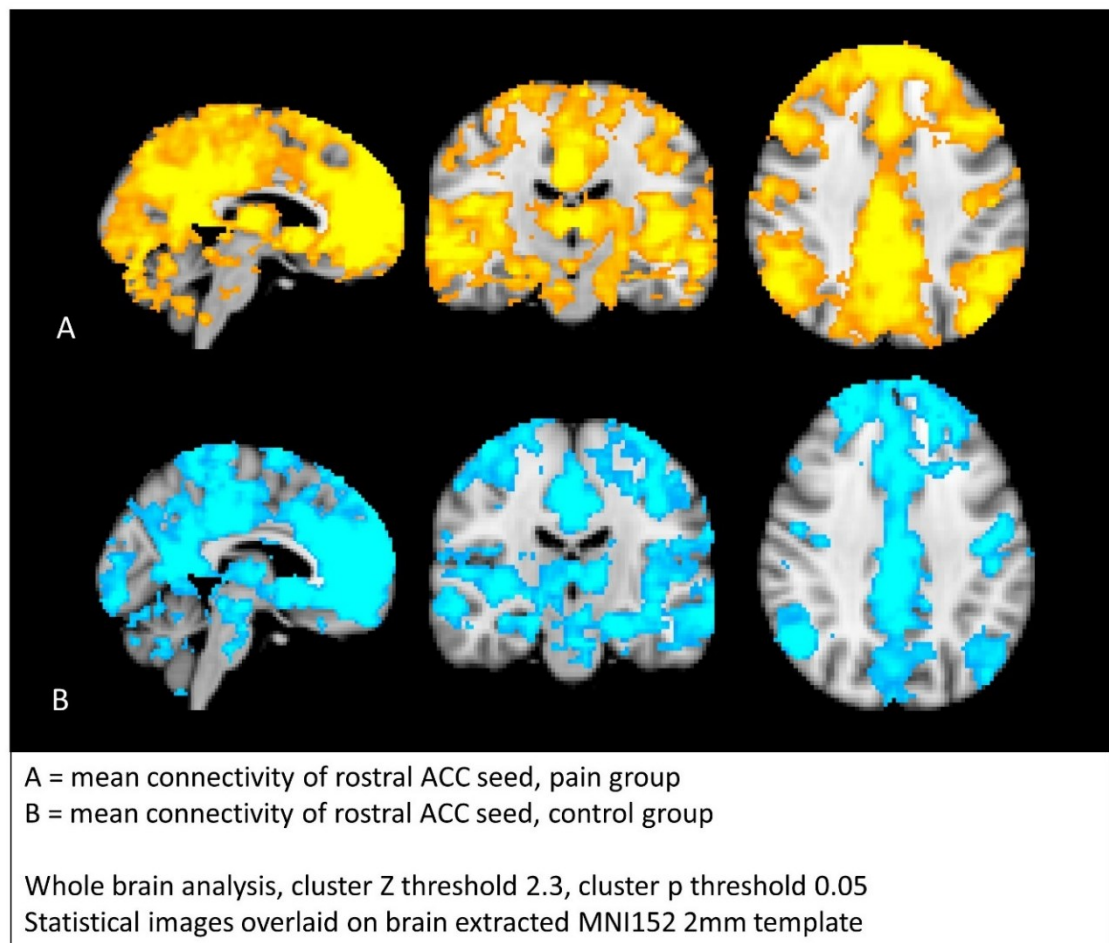
6.3.2 Mean ACC seed connectivity by group

Mean ACC seed connectivity for the pain group and control group, calculated separately, are shown below (Figure 32). Positive correlations with rACC timecourses are shown. No negative correlations were found.

In both groups, functional connectivity of the rACC seed with a bilateral distributed network of cortical and subcortical structures including frontal and prefrontal cortices was observed. Functional connectivity was centred on grey matter (as expected). Little correlation with CSF or WM timecourses was observed.

Both groups showed functional connectivity with frontal cortical structures thought to be important in the appraisal of painful stimuli (see Chapter Four, page 119) including frontal pole and dorsolateral prefrontal cortex. Parietal cortices, anterior and posterior cingulate cortices, bilateral thalamus, sensory cortices (postcentral gyri), cerebellum and brainstem (the latter including midbrain, pons and, in the case of the pain group, rostral medulla) were shown to be functionally connected to the rostral ACC seed. Images are shown for qualitative comparison (Figure 32).

Figure 32: Mean functional connectivity of rostral ACC seed, separately calculated in pain and control groups



6.3.3 Associations of the presence of pain

Unpaired t-test: whole brain level

At the whole brain level, no statistically significant difference in functional connectivity of the rACC seed was found ($z=2.3$, $p \leq 0.05$, whole brain analysis).

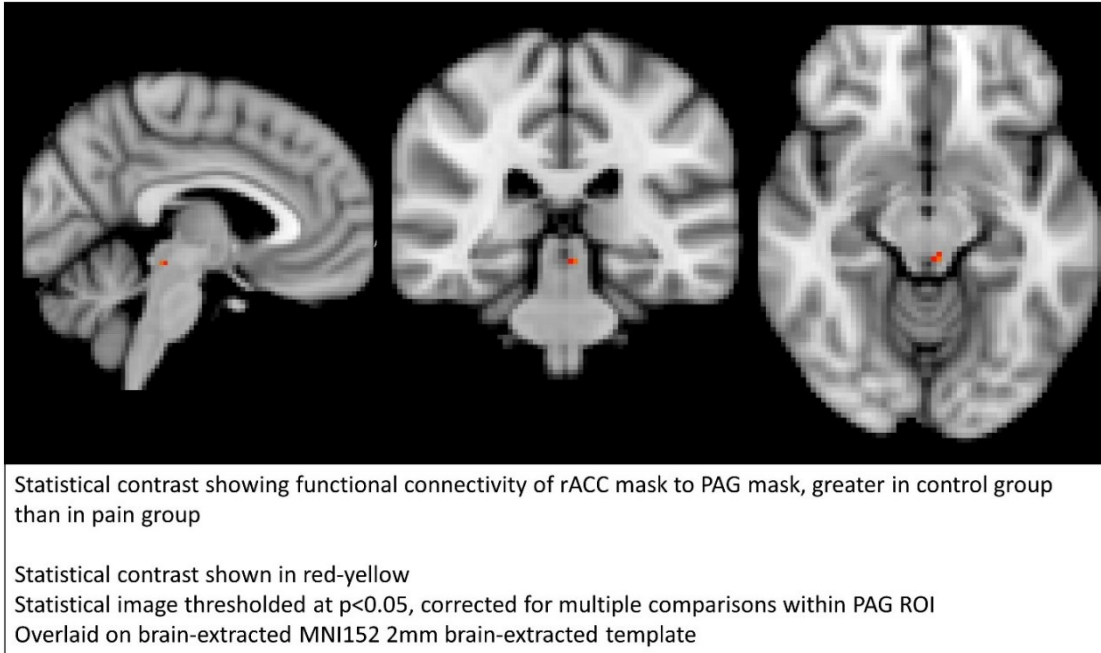
Unpaired t-test: PAG spherical ROI

In a small volume comparison focussed on the periaqueductal grey matter (using the mask described above - 6.2.4), a statistically significant difference in rACC:PAG functional connectivity was observed. Specifically, functional connectivity between the rACC seed and left caudal PAG was higher in the control group, than in the pain group. No functional connectivity was found to be higher in the pain group, than in the control group ($p \leq 0.05$, threshold free cluster extent, corrected for multiple comparisons within PAG ROI) (Figure 33).

Functional imaging associations of neuropathic limb pain in MS

Maximal differential connectivity was observed at MNI coordinates $x = -4$, $y = -32$, $z = -12$.

Figure 33: Differential functional connectivity between ACC seed and PAG, in pain and control groups

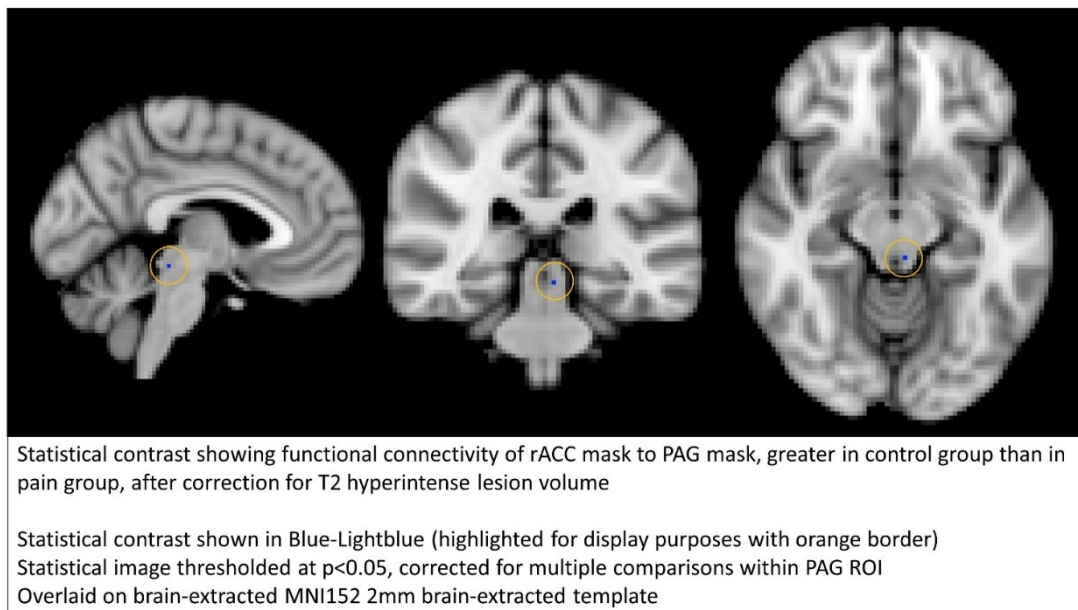


Functional imaging associations of neuropathic limb pain in MS

Unpaired t-test: PAG spherical ROI after correction for T2 hyperintense lesion volume

After inclusion of T2 hyperintense lesion volume as a covariate of no interest, rACC:PAG functional connectivity was only found to be statistically significant at a single voxel. This should be interpreted with caution but may demonstrate a diminution of previously observed associations, when T2 hyperintense lesion volume is included in the model as a covariate of no interest (Figure 34, and compare to Figure 33).

Figure 34: Differential functional connectivity between ACC seed and PAG in pain and control groups, after correction for T2 hyperintense lesion volume



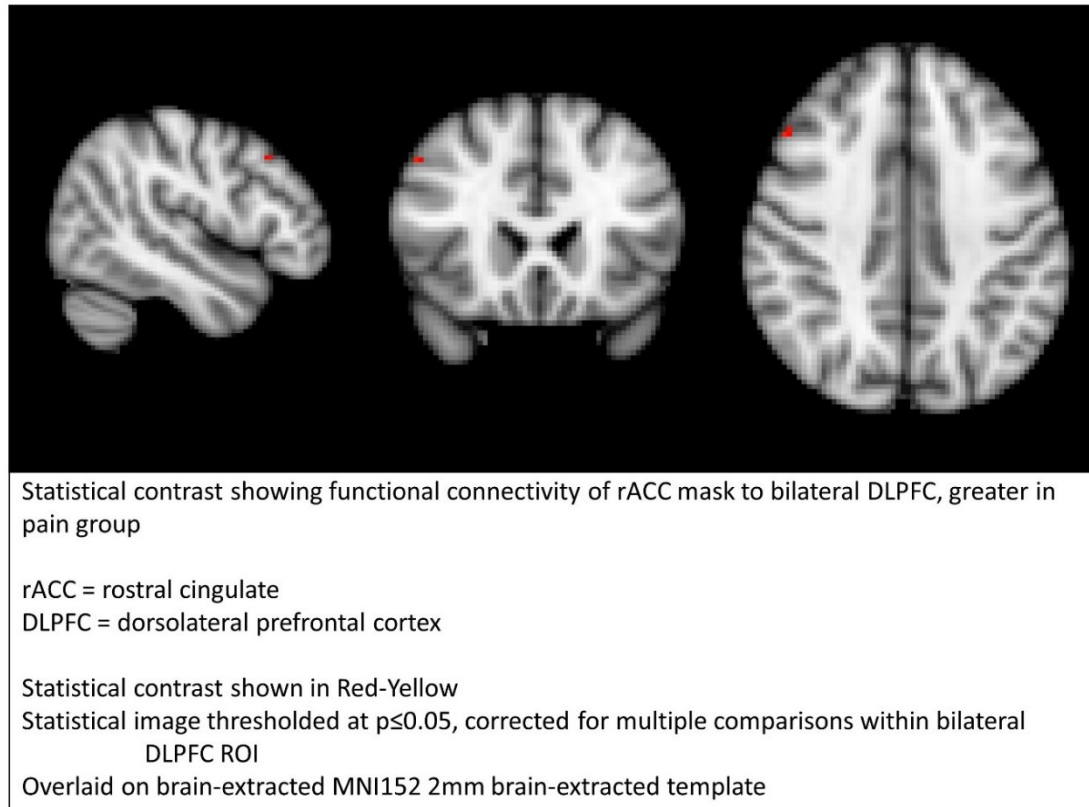
Unpaired t-test: bilateral DLPFC region of interest

In a small volume comparison focussed on the bilateral dorsolateral prefrontal cortex (using the mask described above - 6.2.4), a statistically significant difference in rACC:DLPFC functional connectivity was observed. Specifically, functional connectivity between the rACC seed and right DLPFC was higher in the pain group, than in the control group. No functional connectivity was found to be higher in the control group, than in the pain group ($p \leq 0.05$, threshold free cluster extent, corrected for multiple comparisons within DLPFC ROI) (Figure 35).

Functional imaging associations of neuropathic limb pain in MS

Maximal differential connectivity was observed at MNI coordinates $x=50$, $y=22$, $z=40$ (middle frontal gyrus (116)). The area of differential connectivity overlapped with the spherical DLPFC ROI specified by Eippert and colleagues (110).

Figure 35: Differential functional connectivity between rostral ACC seed and bilateral DLPFC region of interest, between pain and control groups



Unpaired t-test: bilateral DLPFC region of interest after correction for T2 hyperintense lesion volume

After inclusion of T2 hyperintense lesion volume, no statistically significant differential connectivity of rACC seed and bilateral DLPFC region of interest was found.

Unpaired t-test: “control” region of interest, occipital cortex

No statistically significant differential functional connectivity between rACC seed and occipital region of interest was found.

Functional imaging associations of neuropathic limb pain in MS

6.3.4 Associations of the severity of pain

Regression analysis: whole brain

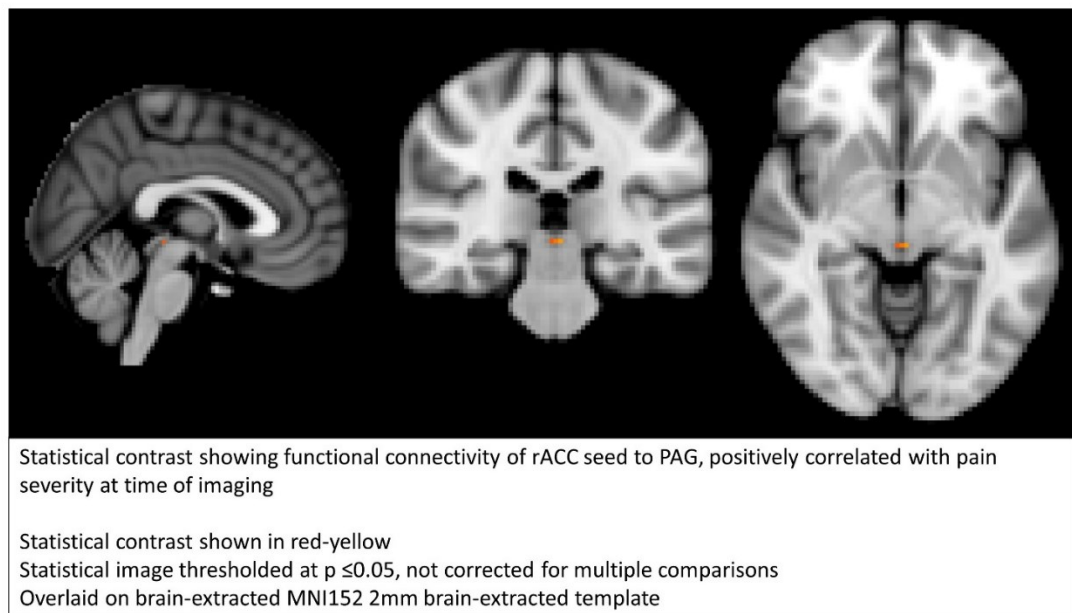
No association between rACC connectivity and pain severity rating at the time of imaging was observed at the statistical threshold applied ($z=2.3$, $p \leq 0.05$).

Regression analysis: PAG region of interest

No statistically significant association between rACC connectivity and pain severity rating at the time of imaging was observed (TFCE corrected $p \leq 0.05$).

A trend to positive association between pain severity and rACC:PAG functional connectivity was noted at the statistical threshold TFCE $p \leq 0.05$, not corrected for multiple comparisons. Maximal correlation was observed at MNI coordinates $x = -2$, $y = -28$, $z = -6$ (Figure 36). No trend to negative association between pain severity and rACC:PAG functional connectivity was noted at the same threshold (Figure 36).

Figure 36: Trend to positive correlation of rostral ACC: PAG connectivity, with pain severity at time of imaging



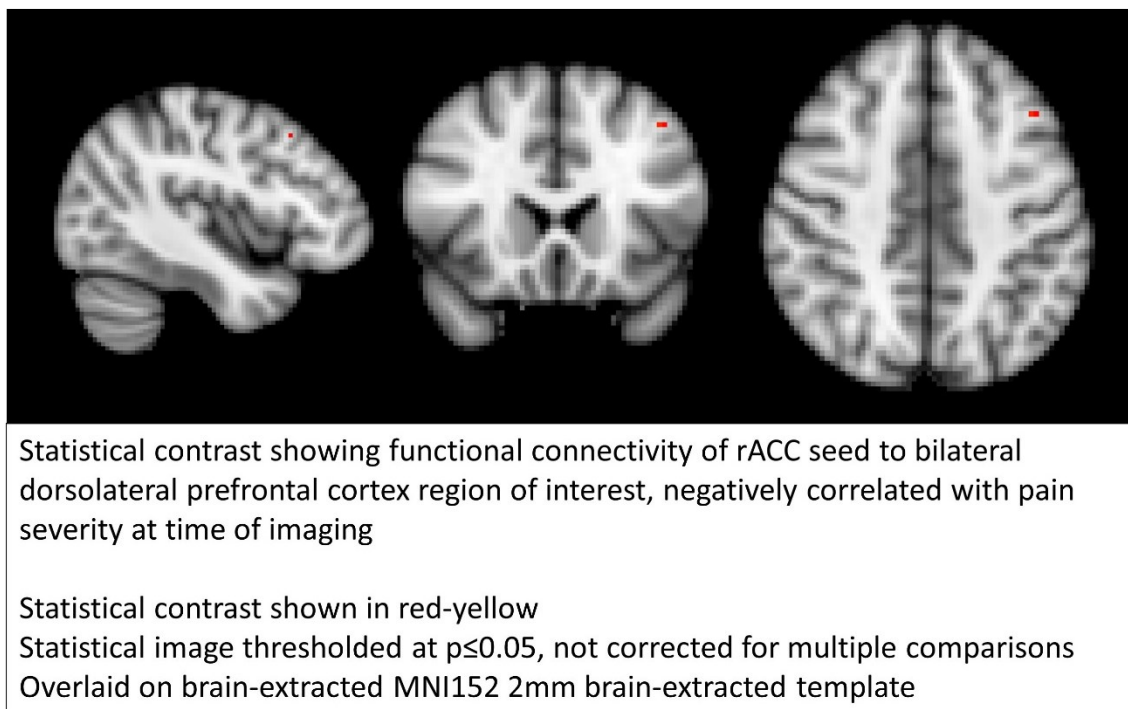
Regression analysis: DLPFC region of interest

No statistically significant association between rACC connectivity and pain severity rating at the time of imaging was observed (TFCE corrected $p \leq 0.05$).

Functional imaging associations of neuropathic limb pain in MS

A trend to negative association between pain severity and rACC:DLPFC functional connectivity was noted at the statistical threshold TFCE $p \leq 0.05$, not corrected for multiple comparisons. Maximal correlation was observed in the left middle frontal gyrus (116) at MNI coordinates $x=-40, y=20, z=44$. No trend to positive association between pain severity and rACC:DLPFC functional connectivity was noted at the same threshold (Figure 37).

Figure 37: Trend to negative correlation of rostral ACC: DLPFC connectivity, with pain severity at time of imaging



Regression analysis: “control” region of interest, occipital cortex

No association of functional connectivity between the rACC seed and occipital region of interest was found with pain severity (in either positive or negative direction, including at a more relaxed statistical threshold of $p \leq 0.05$ uncorrected for multiple comparisons).

6.3.5 Associations of T2 hyperintense lesion volume

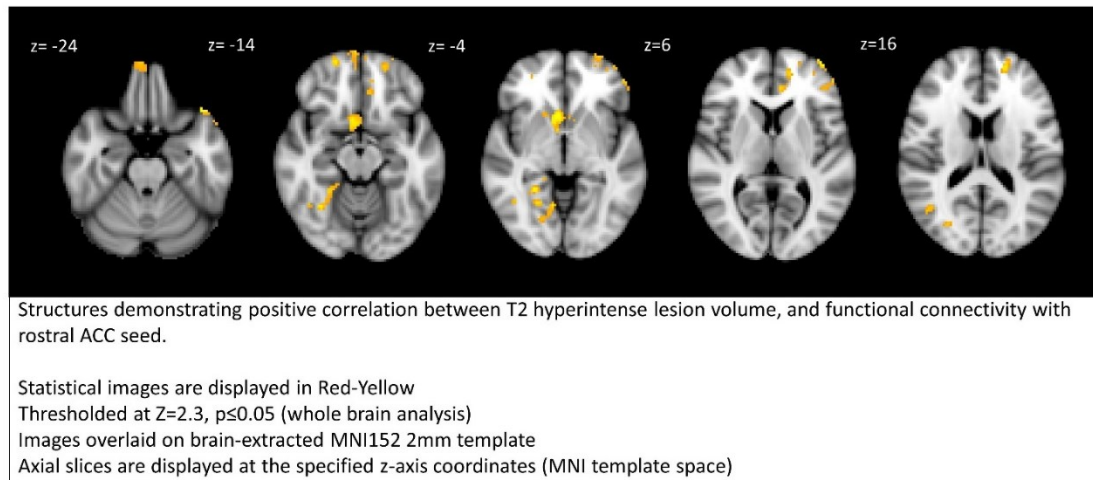
A positive correlation between T2 lesion volume and rACC seed functional connectivity to various regions including cortical and subcortical structures was observed.

No negative correlation between T2 lesion volume and rACC seed functional connectivity was observed. Please see below (Figure 38, Table 50).

Table 50: Structures manifesting higher functional connectivity to ACC seed in correlation with increasing T2 lesion volume

Cortical structures	MNI coordinates		
	x	y	z
Occipital cortex (right)	38	-76	28
Frontal pole (bilateral)	18	64	-10
	-20	56	-14
Nucleus accumbens (right)	8	14	-8
Nucleus accumbens (left)	-6	14	-8
Caudate head (right)	10	12	0
Temporal pole (left)	-50	14	-20
Paracingulate gyrus/anterior cingulate cortex (left)	-6	38	-10
Orbitofrontal cortex (left)	-44	26	-16

Figure 38: Structures demonstrating positive correlation between T2 hyperintense lesion volume, and functional connectivity with rostral ACC seed



6.4 Discussion

This small cross-sectional seed-based resting state fMRI study provides first, early, evidence, that functional connectivity of the descending pain modulatory system could be disrupted in people with neuropathic limb pain in relapsing remitting MS.

Functional connectivity of the dorsolateral prefrontal cortex, rostral anterior cingulate cortex and periaqueductal grey matter has been examined. Statistically significant differences in functional connectivity between the rACC seed and PAG, and separately rACC seed and DLPFC, were found between groups. MS lesion volume may contribute to this differential connectivity. Further exploration of the interface between structural and functional connectivity could include diffusion imaging to further explore any role of structurally disrupted white matter tracts.

Please see the Thesis Discussion (page 271) for a detailed discussion of study findings, implications and methodology.

Chapter 7 Participant experience of the study

7.1 Introduction

The experience of participants in this study was important. In particular, at the study design stage, it was important to estimate how well subjects might tolerate the proposed study (and its component parts). Component parts of the study included questionnaires, neuropsychological assessment, structural MRI, and fMRI including painful stimuli (all in the presence of a pre-existing central nervous system disease).

When designing the study, estimates of anticipated participant experience formed part of an important balance between optimising participants' experience of the study, and optimising scientific utility of the study. There is an ethical duty to minimise distress during study participation (297). Participant distress, however, should also be balanced against unnecessary restriction of study activities, if the study protocol was in fact well tolerated (297). Some previous studies of participant experience have found that researchers have significantly overestimated the negative effects of taking part in research, and underestimated any positive effects (298).

I planned to base the study design on published evidence around participant experience of research studies, where available. Little published data, however, was available to guide estimates of participant experience. Study design choices (for instance acceptable duration of MRI protocol) were largely made on discussion with experienced colleagues, and based on their personal experience.

7.1.1 Rationale for survey of participant experience

I felt that it would be useful to formally assess subjects' experience of the current study. There were several reasons for this.

- Firstly I felt that the participants' experience of the study was of intrinsic interest.
- Furthermore, detailed assessment of their experience might reflect
 - overall experience of the study,
 - experience of particular aspects of the study

References

- including opportunity to identify aspects which could be improved for future studies, and
- motivation to undertake further studies.

Importantly, this information might also inform development of any future studies of this type, with particular reference to likely participant tolerance of the proposed protocol. Knowledge of participants' experience might also help to inform research ethics committees (299).

7.1.2 Aims

- 1) To identify any existing questionnaire or survey instruments validated to establish participant experience of research studies
- 2) If needed, to construct an instrument for this study
- 3) To assess participant experience of the study using this instrument
- 4) In addition I also sought to establish a pool of study participants who would be willing to be contacted to
 - give advice on design of future studies at, or closely associated with, the Anne Rowling Regenerative Neurology Clinic (ARRNC) and
 - Participate in future studies at, or closely associated with, ARRNC.

7.2 Methods

7.2.1 Ethical permissions

All methods described were approved by the relevant medical ethics committee

7.2.2 Identifying existing instruments

I searched the existing literature for validated questionnaire or survey instruments which assess participant experience of research. I searched Google Scholar using combinations of the terms “participant” “experience” “satisfaction” “feedback” “survey” and “questionnaire”, and searched citation records of relevant articles. I also spoke to specialists in patient/public engagement and in relevant fields (neuroimaging, neuropsychology, pain research, neurology research).

7.2.3 Survey design process

A survey instrument was designed by PF, in discussion with others (including Allison Worth, Shuna Colville and Denise Cranley).

Priorities were identified as simplicity, brevity, and assessment of experience of the study overall, as well as its component parts.

A first draft was written by PF, circulated for comment and amended as required.

Using the established study newsletter (see Appendix: Study Newsletters) study participants were also invited to comment on topics to be included in the survey.

7.2.4 Survey design

The survey was divided into sections reflecting the design of the study. These included

- Exchange of information about the study, prior to participation
- Overall rating of satisfaction
- Assessments in Anne Rowling Regenerative Neurology Clinic (ARRNC)
- Assessments at Clinical Research Imaging Centre (CRIC)
- Motivations and further comments
 - Including motivation for taking part, positive and negative aspects of the current study, aspects which could be modified for future studies.
- Feelings about participation in future discussion or research

Scales of 0 to 7, where a single numerical rating had to be selected, were used for quantitative feedback. Anchors of “1 – not at all” and “7-completely” were used throughout. Space for unrestricted qualitative feedback (free text, without word limit) was given at the end of each section.

7.2.5 Delivering the survey

The survey was delivered online using Bristol Surveys (www.onlinesurveys.ac.uk). Access to the survey was password-protected, using the same password for all participants. Participants did not have access to results.

Where survey participants requested a written rather than online copy of the instrument (n=1), a PDF of the survey was printed out using inbuilt tools in Bristol Surveys, and posted to the participant with a stamped-addressed envelope (SAE).

Survey participants were specifically requested not to input name, address, or other personal information during completion of the survey.

All questions within the survey were optional, apart from the overall rating of satisfaction.

Survey participants were told that their feedback was anonymous. They were given the option of enclosing their study ID number (included in the invitation to participate) which could be linked to their anonymised study data, but not to link their identity to their responses.

7.2.6 Invitation to participate

Upcoming delivery of the survey was highlighted using the study newsletter (see above) in order to maximise awareness.

First invitation to participate was by written letter, giving a URL for the survey as well as email and phone contacts, and offering to send out paper copies of the survey (with SAE) to any participants who would prefer this. Each participant's unique study identifier number was included in the invitation, and they were asked to input this number at time of completion of the survey if they wished.

Second invitation was by email, including the URL in the email, so that the link could simply be clicked rather than typed into the browser. Emails were only sent to participants who had already given permission to be contacted by email, and where email contact had been established with the participant themselves (in one case email contact was largely with the participant's spouse, and an email was not sent to this participant).

A last reminder was included in a subsequent study newsletter.

References

7.2.7 Analysis

Quantitative data

For numerical ratings of satisfaction, data distribution was assessed for Gaussian distribution by inspection of data histograms and Quantile-Quantile (QQ) plot.

Where distribution was felt to be non-Gaussian, median and interquartile range were reported as summary measures. Boxplots were constructed. No data transformations were used. The Wilcoxon rank-sum test (Mann-Whitney U test) was used to test significance level of differences between groups.

For binary variables (for instance gender), a 2x2 table was generated. The Chi-squared test was used for larger samples, and the Fisher exact test for smaller samples. The Fisher Exact test was used where overall total of the table was less than 20, or where the overall total was between 20 and 40, and the smallest of the four expected numbers was less than 5 (137). Alternatively the Fisher Exact test was used where Chi-squared analysis output from R identified the possibility of an inaccurate estimate because of small sample numbers.

Significance was assigned at the 5% level, unless otherwise stated. In this exploratory analysis, no correction for multiple comparisons was applied.

Qualitative data

For the purposes of this thesis, qualitative data has been subject to informal analysis only.

For comments on specific component parts of the study, text feedback was reviewed by PF, themes identified and a sample of representative quotes is reproduced.

For comments on motivation to take part in research, where a larger volume of feedback was available, the text feedback was reviewed repeatedly by PF. General themes were identified. Statements of motivation within each participant's free text feedback were identified, and grouped into general themes. For illustrative purposes, the number of statements falling into each theme were identified. This informal analysis forms an initial step in intended further formal thematic analysis (300, 301).

References

7.3 Results

7.3.1 Existing instruments

I did not find any validated assessment instrument aiming to measure participants' experience of research experience. This is in keeping with previous studies of treatment research, where no pre-existing instrument was found (298).

7.3.2 Participant input into survey design

Two research subjects made suggestions for survey design. These included inclusion of a question on perceived relevance of the study instruments for each participant. A question on this topic was therefore included in the survey.

7.3.3 Survey Uptake

30 subjects completed the survey overall. 12 responded to the first invitation (letter), 17 to the second (email), and 1 to the last (study newsletter). No participant described difficulty in accessing or completing the survey.

7.3.4 Demographics

Of thirty respondents, median age (years) was 40.5 (IQR 35.00 to 51.25), 27 of 30 (90%) were female, median EDSS was 1.50 (IQR 1.00 to 2.88) and 21 of 30 (70%) related chronic pain at the time of study participation.

7.3.5 Comparison of survey responders and non-responders

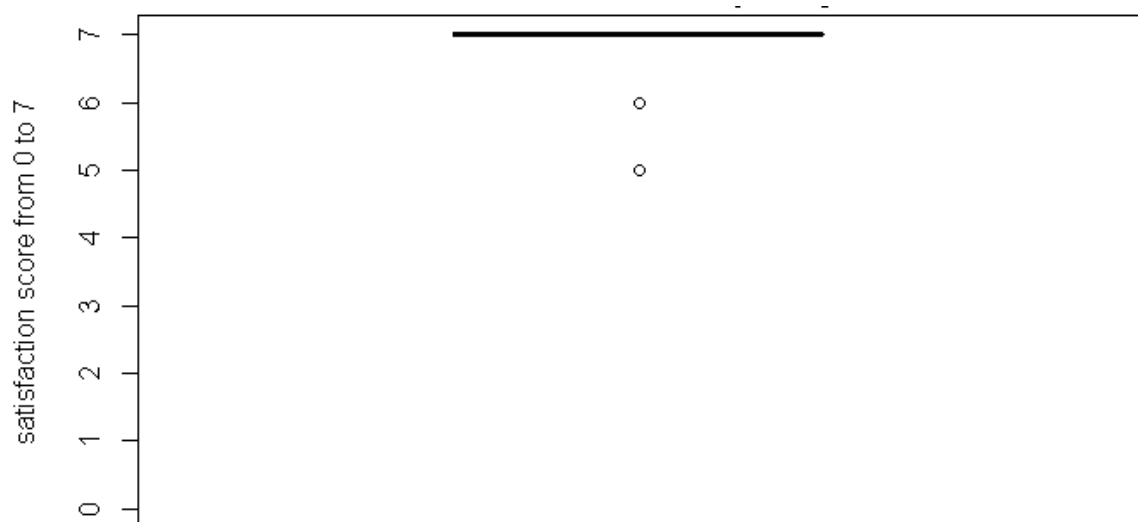
Survey responders were significantly less disabled than non-responders (Median EDSS 1.5 vs 2.0 in non-responders, $p=0.04$). There was a trend towards a higher proportion of females in survey responders which did not attain statistical significance ($p=0.054$). Age and presence of chronic pain did not vary significantly between responders and non-responders. Please see Table 51 below.

Table 51: Comparison of demographics, survey responders and non-responders

	Responders	Non-responders	"p" value
Age (years) (median,IQR)	40.5 (35.0 to 51.2)	41.0 (35.5 to 43.2)	0.73
Gender (percentage female)	27/30 (90%)	11/17 (65%)	0.054
Disability (EDSS) (median,IQR)	1.50 (1.00 to 2.88)	2.00 (2.00 to 5.50)	0.04
Chronic pain present (percentage)	21/30 (70%)	10/17 (59%)	0.65
IQR = Interquartile Range			

7.3.6 Overall satisfaction with study participation

Participants were asked "How satisfied, overall, were you with your experience of taking part in the MS sensation/pain study?" Median satisfaction rating (0 = "not at all satisfied", 7 = "completely satisfied") was 7.0 (IQR 7.0 to 7.0). Please see Figure 1 below.

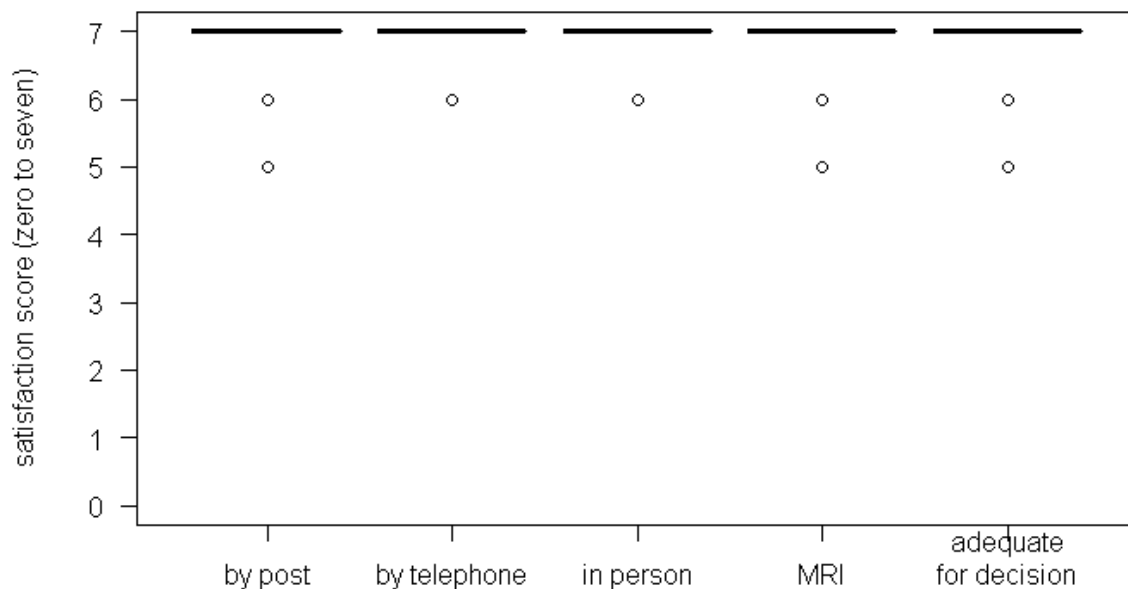
Figure 39: overall satisfaction with participation (0= "not at all", 7 = "completely")

7.3.7 Information exchange and discussion

Participant satisfaction was high for information given by post prior to participation (median 7.0, where 0 is not at all satisfied, and 7 completely satisfied), by telephone prior to first visit (median 7.0), in person at first visit (median 7.0), and specifically regarding explanation of MRI procedures (median 7.0). Participants also felt that information given was adequate and appropriate to make a decision on study participation (median 7.0).

Please see figure 2 below.

Figure 40: satisfaction with information given (0 = "not at all", 7 = "completely")



Free text comments

In general, feedback on information exchange was positive. One respondent felt that they were given too much information.

One respondent highlighted that her previous incidental coil embolization procedure had not precluded clinical MRI in the past (at 1.5T), and therefore she did not declare it at time of study entry. In this participant's case, the situation was discussed with the CRIC physics team, who felt that imaging at 3T might be associated with risk, and therefore imaging was not carried out.

References

“well conducted and all questions relevant”

“In my personal opinion the word “pain” might be misleading. One might worry that it is going to be a traumatic experience (which it isn’t)”

“Everything was very well explained and it was clear that informed consent was considered the most important thing”

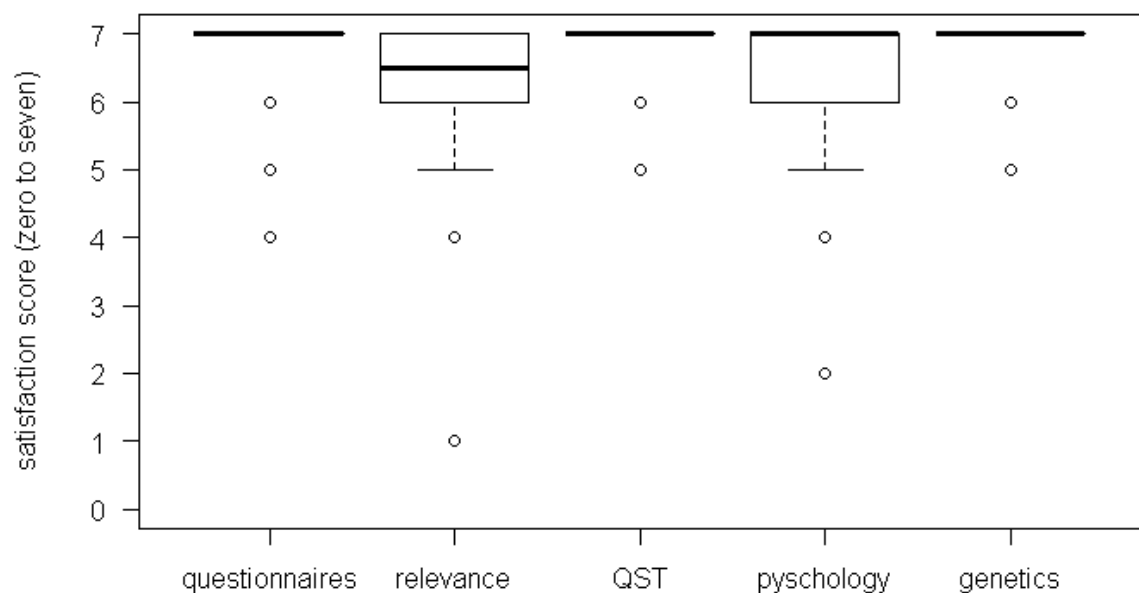
“There was more information than I required”

“I had some metal in my kidney (*sic*)...but as I had had an MRI in another hospital... I assumed it would be OK... I did not know...it was not advisable”

7.3.8 Study procedures carried out at Rowling Clinic

Separate ratings were obtained for questionnaires, perceived relevance of questions asked to each individual, Quantitative Sensory Testing (QST), neuropsychological testing and genetic testing (in most cases venepuncture, in two cases obtaining saliva sample). Median satisfaction score was 7.0 for all ratings, apart from perceived relevance (median 6.5). Please see figure 3 below.

Figure 41: satisfaction with assessments at Rowling Clinic (0 = "not at all", 7 = "completely")



Free text comments

Free text feedback on neuropsychology assessment, in particular, was mixed (though overall quantitative feedback was positive, as detailed above). Some participants related finding the neuropsychology testing demanding or stressful, though others enjoyed it.

“I found it all very fascinating and (PF) made it a pleasant experience”

“Felt really stupid in the psychology part, but made me feel really at ease with the situation so really helped me to relax”

“Found the psychology testing very emotional and exhausting”

“Thoroughly enjoyed the psychology testing! Very interesting. Would like to know more about this”

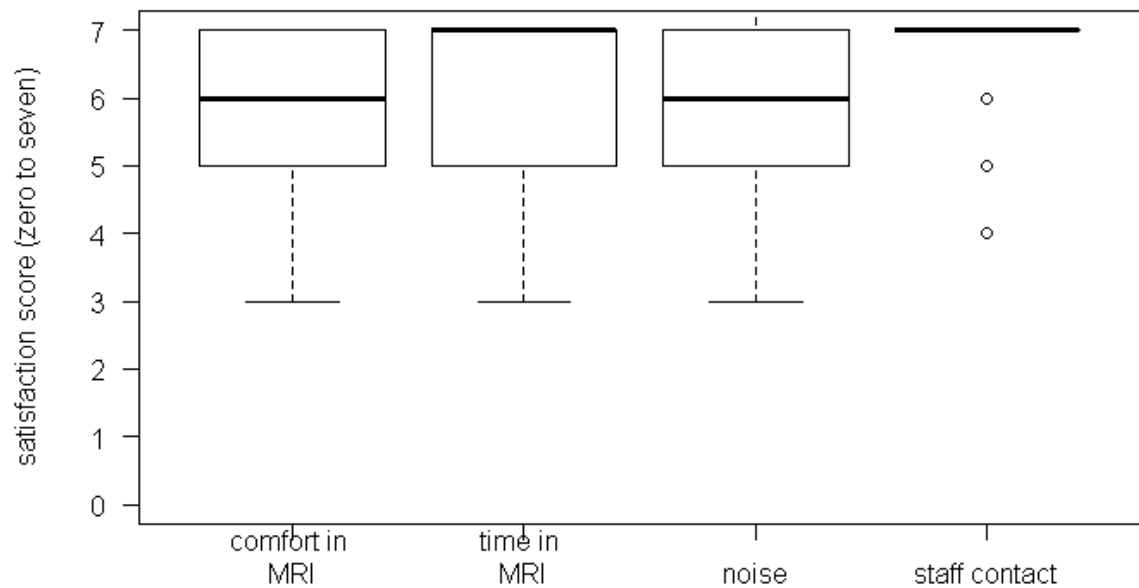
“Well conducted”

7.3.9 MRI imaging at Clinical Research Imaging Centre (CRIC)

Median satisfaction rating was 6.0 for comfort in MRI scanner, and noise in MRI scanner. Median rating was 7.0 for time in MRI scanner, and contact with staff during MRI procedure.

Please see figure 4 below:

Figure 42: satisfaction with aspects of MRI imaging at CRIC (0 = "not at all", 7 = "completely")



Free text comments

Several participants related feeling cold during MRI imaging. Others related feelings of claustrophobia, or discomfort, but that they were able to overcome these, and in some cases were specifically glad that they did so.

“Was really nervous about getting the scan, but everyone was lovely and made me feel really comfortable and at ease”

“Quite cold in MRI room”

“Low scores on a few of the questions ...simply because I am extremely claustrophobic and was very anxious about being in the scanner. Staff were very patient, kind and helpful, no pressure to continue if I couldn’t do this part of the research and knew I could have stopped at any point. Pleased I did it!”

“It made a huge difference having a mirror attached to the headpiece so I could see out. I hadn’t had this before and it made it much easier”

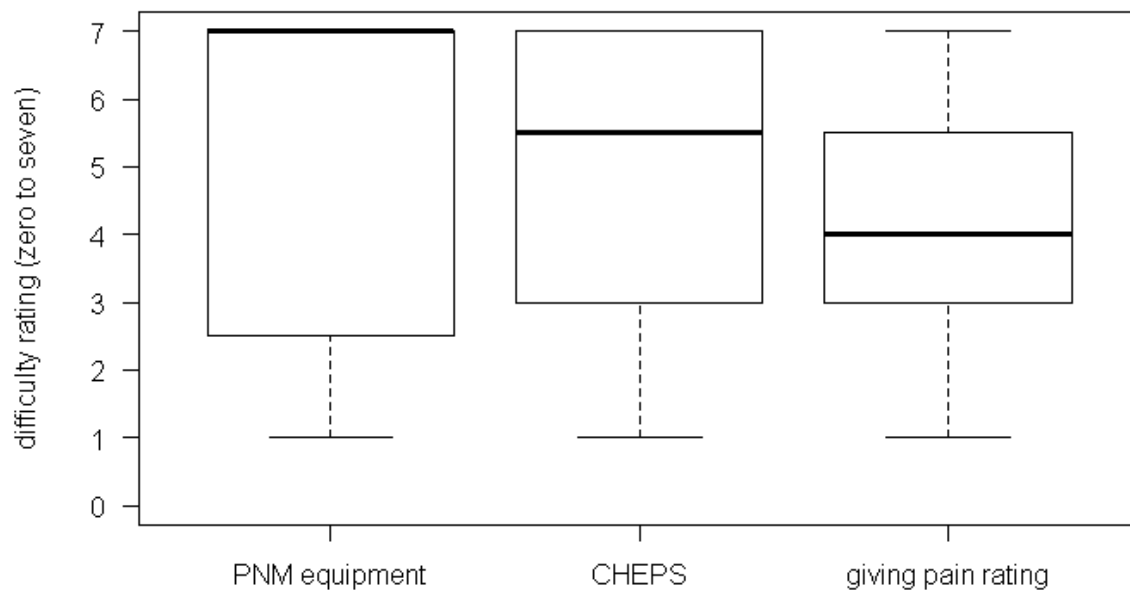
7.3.10 fMRI procedures

Satisfaction ratings were given for difficulty associated with physiological noise monitoring equipment (“how difficult did you find it to have the fingerclip and the thin cable round your lower chest, while in the scanner?”), noxious thermal stimuli during fMRI (“how difficult did you find it to experience the actual heating at the ankle, while in the scanner?”) and also giving numbers for pain ratings (“How difficult did you find it to give numbers to estimate pain or discomfort during the scan?”).

Please note that ratings used in this section were different to those used previously, in that ratings from 0 “not at all difficult” to 7 “completely difficult” were used, in contrast to the ratings described above.

Median rating for PNM equipment was 7.0, for noxious heat administration 5.5, and for giving a pain rating 4.0. Please see figure below.

Figure 43: perceived difficulty associated with functional MRI procedures and equipment. (0 = "not at all ", 7 = "complete ")



Free text comments

Several participants mentioned that they found it difficult to estimate their own pain levels during imaging. No participants made specific comments regarding the PNM equipment.

Some participants implied a comparison with others in their pain responses (despite subjects being specifically advised that their pain responses were highly individual).

“It’s always difficult to allocate numbers to something subjective as you have no reference scale”

“Difficult to have a subjective view to estimate pain...am I being tough or am I a complete wimp???”

7.3.11 Motivation to participate in research

29 free text comments were obtained. Some of these contained more than one reason for participation. Overall 39 statements of motivation were extracted

26 of these described a wish to help others, often specifically others with multiple sclerosis.

I decided to take part in the study as I feel that the more is understood about this condition, the better it is for everyone who suffers from it.

I took part in study as my Ms pain has gradually gotten worse over time and it's a real struggle everyday with the pain I experience and hard to explain how I feel most days cause even me myself can't understand some of the pain/sensations I get so would do anything to help make more sense of things for others and myself for the future and hopefully one day maby with the research found might be able to ease some of the pain for myself and others

Anything to help the understanding or potential treatment of MS

Wish to help the study. Interested to know how my experiences compare to others.

References

Because it is about the only thing that I can do to help with finding new treatments for this disease and because the drugs I take had to be tested on a previous cohort-you have to 'play it forward'

Anything that helps provide more information to guide treatments for MS is a worthwhile cause

Four mentioned personal interest, though this was mentioned as a motivator for participation, only in combination with other factors.

...out of interest...

...I was interested in learning more about the study...

Four mentioned a desire to help themselves in some way

I would like to think I was perhaps helping myself or others...

Any research into ms is a good thing for me and my family (...)

Three mentioned a desire specifically to “give back” to NHS or ARRNC staff, in return for care received.

The Ann Rowling Clinic has been fantastic, very supportive and friendly every visit I have there. I would be happy to do take part in any study for them ...

Having received excellent care and treatment from the NHS Neurology Dept and Anne Rowling Centre I want to give something back to show my gratitude.

One mentioned a desire to obtain an up to date MRI scan.

(...) and I also knew it would put an up to date scan on my medical records

One mentioned guilt around resources expended on their care through the NHS, and implied that they felt a need to repay this.

I think like many MS sufferers you feel a bit guilty about the amount of NHS resources pumped into your care and medication, this is a small way of contributing (apart from via the tax system obviously).

7.3.12 Changes suggested for future studies

Some participants suggested specific changes, including weekend attendance (n=1), or payment for participation (n=1).

Some participants mentioned that they would like individual feedback on their imaging results, although individualised feedback was provided to all participants by post. All participants were supplied with a formal written comment on their MRI imaging results, and result of imaging posted to GP, though these comments may have been made before this information was received by the participant.

Taking time off work to attend can be an issue-weekend attendance could make that possible although I appreciate may not be possible

I guess because it is quite subjective, it was sometimes a bit tricky to judge the pain levels from the heat device. Not sure how exactly it could be improved though, sorry!

Tighten up on info re metal in body and explaining that research scanner is more powerful than hospital ones so even if a participant has had MRI since acquiring metal, best to double check suitability for MRI as part of research study.

I cannot think of anything. I think it was conducted extremely well

It will be good if we, as patients can get any update on our individual status or any findings during the test.

I think a token payment or gift card for the time would have been a nice gesture.

Very satisfied with professional yet personal format of this study. I felt that my experiences and feelings were important, nothing intrusive and everything explained well so no need to change anything from my point of view.

7.3.13 Any specific strengths of study

Some specific comments included positive feedback on the study newsletter, and on encounters with staff during the study in general (including ARRNC and CRIC).

I have really appreciated the newsletter update.

I found it really positive to no that I'm not the only one living with this pain and that every case is different and that there are people out there trying to help find cures for this

The whole thing was really interesting and every person I spoke to was excellent. The mirror in the scan was the highlight ! - tell people about this beforehand

(PF) was very easy to get on with and extremely helpful. Made the experience very worth my while. Hope it helps.

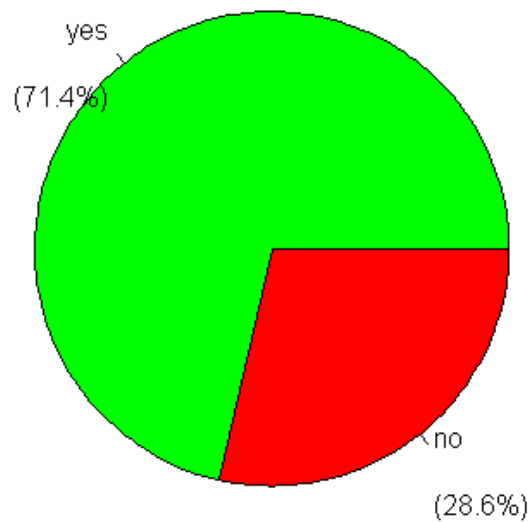
I would like to express my thanks to the professional and caring attitude of all the members of staff I met during the study.

It was very interesting to take part and to learn more about the research that is happening and gain a greater understanding of the disease

7.3.14 Considering contribution to future study design

71.4% of respondents stated that they would be interested in being contacted about giving advice on how future studies (at ARRNC or closely related facilities) might be designed (see figure below). All of these respondents consented to be included in a database held at ARRNC for these purposes.

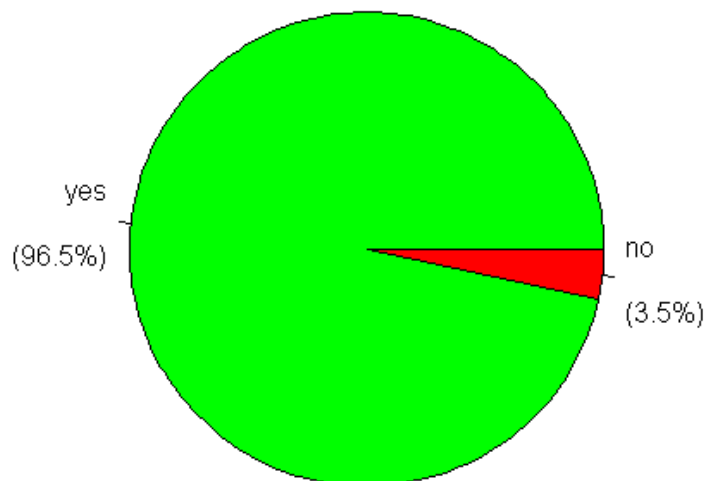
Figure 44: Would you be interested in being contacted about giving advice on how future studies might be designed? (n=29)



7.3.15 Considering participation in a future study

96.5% of respondents stated that they would be interested in taking part in a similar study in the future. (see figure below). All of these respondents consented to be included in a database held at ARRNC for these purposes.

Figure 45: would you consider taking part in a similar study in the future? (n=30)



7.4 Discussion

The assessment of participant experience in this study provides early information which could help guide researchers designing or executing future similar studies.

Some issues inherent in the acquisition of this data, which are relevant to possible biases and could influence results, and conclusions drawn from these results, are identified.

Please see Chapter 8: Discussion for a more detailed coverage of related issues.

Chapter 8 Discussion

8.1 Overview

In this thesis the following work is described:

- Systematic Review and Meta-Analysis investigating prevalence, associations, and natural history of pain syndromes in adults with multiple sclerosis
- Systematic Review of existing neuroimaging studies of pain syndromes in adults with multiple sclerosis

And a prospective case-control study of adults with relapsing remitting multiple sclerosis, with and without neuropathic limb pain, specifically addressing the following aspects:

- Clinical, behavioural and cognitive associations of neuropathic limb pain
- Structural neuroimaging associations of neuropathic limb pain
- Functional connectivity of the descending pain modulatory system in neuropathic limb pain

In addition, a study of participant experience is described.

8.1.1 Restatement of main study hypothesis

As discussed, the overarching hypothesis of the prospective clinical study was that:

In adults with relapsing remitting MS, dysfunction of the descending pain modulatory system will be associated with the presence, and severity, of neuropathic limb pain.

The first two experimental chapters provide systematic review evidence to inform design of the prospective clinical study.

The third, fourth and fifth chapters address this hypothesis in a prospective case-control clinical and neuroimaging study.

References

8.2 Findings

Findings are discussed as relevant to each of the experimental chapters in turn.

8.2.1 Systematic Review: Prevalence, associations and natural history of pain in multiple sclerosis

Prevalence of pain, and pain syndromes

Pain in MS was found to be common, affecting around 63% of adults with the condition (95%CI 55% to 70%). Individual pain syndromes including headache, extremity neuropathic pain, back pain, painful spasms, Lhermitte's sign, and trigeminal neuralgia were, in turn, all found to be common. These results support previous findings that pain in the MS population is heterogeneous, and includes several pain syndromes and mechanisms.

These findings attempt to quantify the prevalence of these syndromes in the MS population, and suggest that headache, followed by extremity neuropathic pain, are the most common pain syndromes, and trigeminal neuralgia the least common of those investigated.

Considerable heterogeneity is however associated with these estimates, and prevalence of some painful syndromes (in particular optic neuritis) remains unclear.

Investigation of heterogeneity by meta-regression did not identify any of the tested demographic variables as significantly associated with the presence of pain, after Bonferroni correction for multiple comparisons (Table 6: Meta-regression analysis of study- and population- level associations with pain prevalence estimates). Tested variables were EDSS, proportion of female gender in population, proportion with progressive MS in population, and disease duration. On testing of study methodology variables, presence or otherwise of investigator blinding, study of inpatient vs outpatient population, and pain timeframe (whether pain was assessed within the last month or longer than this) were assessed. Only pain timeframe significantly explained estimate heterogeneity, and only within the headache prevalence studies.

Although both neuropathic and nociceptive pain syndromes were found to be common in the described studies, my findings additionally suggest that neuropathic pain mechanisms may be more prevalent than somatic/nociceptive mechanisms.

Estimation of pain prevalence throughout the disease course

In order to study pain prevalence in relation to the MS disease course, characterisation of MS related pain was sought - either at disease milestones, or longitudinally in evolving disease. The natural history of pain in MS was poorly characterised in the identified studies.

Firstly, no studies of pain incidence were found. No prospective studies of overall pain prevalence prior to disease onset were found (notwithstanding potential methodological challenges). In comparison, Vacca and colleagues (151) retrospectively found that headache was present prior to MS onset in 69.7% of MS headache patients, and that MS onset did not modify pre-existing headaches. Given descriptions elsewhere suggesting predisposition to chronic pain, (302) these findings remain of interest.

Only one prospective estimate of pain prevalence at disease onset (25) was found. Despite early recruitment, mean symptom duration was 30.5 months, and pain prevalence of 73.5% could be regarded as an estimate in early disease. Retrospective estimates from the included studies are lower (overall pain range 11% to 21%, headache range 1.7% to 6.7%). (11, 27, 136, 144, 150, 151) However, disease duration (where reported) for retrospective estimates ranged from 10.8 (11) to 23 years, (144) and thus these figures are vulnerable to recall bias. The apparent discrepancy between prospective and retrospective results could suggest that retrospective estimates relatively under-report pain prevalence at disease onset. Optimal data ascertainment methods in pain epidemiology studies remain under investigation. (303)

Pain in MS relapse

Pain associated with relapse has similarly rarely been studied prospectively, despite clear potential clinical relevance. Several studies specifically excluded pain related to MS relapse, (20, 23) or did not separately report this data. (44, 143) One prospective estimate (147) of headache prevalence in relapse was found (prevalence 38.9%), but no prospective studies of overall pain. In comparison, retrospective prevalence estimates ranged from 63% (145) for overall pain, to approximately 1% for headache or for central pain. (133, 144, 150) Katsiari and colleagues report no relationship between several headache subtypes and relapse activity, though methodology is not described.(148) Relapse-associated pain could be highly clinically relevant in informing immunomodulation decisions. The lack of prospective studies, and wide variation in estimates, suggest that further study is required.

How does pain in MS vary with time?

With regards to longitudinal follow-up of pain syndromes, Stenager and colleagues (7) describe increasing prevalence of several pain syndromes with disease progression (initial mean EDSS 3.4). Brochet and colleagues (25) describe a statistically non-significant decrease in pain prevalence in less disabled subjects with early disease (median EDSS 2). Given the limited available data, including only 117 subjects and extending to a maximum of 5 years follow-up, it is not possible therefore to reliably describe any relationship of MS-related pain to disease evolution.

8.2.2 Systematic Review: Neuroimaging correlates of pain syndromes in multiple sclerosis

Firstly issues identified in the existing literature, and then findings described by the literature are discussed.

Study characteristics and methodology

8.2.2.1.1 *Number and quality of studies*

My findings suggest that the number of studies examining neuroimaging correlates of MS pain is relatively low, and that methodology and quality of these studies is variable.

The majority of included articles are case reports or series. Specifically, only seven hypothesis-driven experimental studies were found. Of these studies, one (154) met all quality criteria. The median number of quality criteria met was eight (out of a maximum of twelve). Several aspects of methodology which could be improved in future studies were identified. Specifically, several authors did not fully specify inclusion or exclusion criteria; and did not fully describe the pain assessment methods used.

In all but one of the studies, structural MR imaging of the brain or spine was used, most frequently used to analyse lesion location, or to investigate other structural causes of pain. No studies used volumetric techniques. The description of image acquisition and reading protocols, and investigator blinding in the original studies was, however, often incomplete. It was also not always clear who read and interpreted the images, and only five of seven experimental studies (154, 174, 176, 183, 191) described blinded image interpretation

8.2.2.1.2 *Pain syndromes studied*

All identified studies investigated neuropathic pain syndromes, despite frequent observations in cross-sectional studies that both nociceptive and neuropathic pains are common in MS (approximate prevalence 18% and 29% respectively (76, 304). There was also an emphasis on investigation of headache disorders and facial pain (74% of all studies), in particular Trigeminal Neuralgia.

This emphasis does not closely reflect current knowledge of prevalence of pain syndromes in MS. While headache is thought to affect around 43% of people with MS, Trigeminal Neuralgia is relatively rare (prevalence 3-4% in MS populations). Other pain syndromes including neuropathic extremity pain, back pain, painful spasms and Lhermitte's sign were relatively understudied (304). Some cranial pain syndromes examined in included studies (such as occipital or glossopharyngeal neuralgia (172, 187, 193, 204) are even less common than trigeminal neuralgia.

These observations could suggest that studies identifying neuroimaging correlates of neuropathic pain syndromes in general, and headache or facial pain syndromes in particular, are disproportionately represented by the current literature (305). The reasons for any such discrepancy are not clear, though these may include an emphasis on interventional techniques for trigeminal neuralgia in some studies. No investigators explicitly studied transition from acute to chronic pain states.

8.2.2.1.3 Headache studies

The included headache studies largely aimed to examine neuroimaging correlates of specific headache subtypes. Small studies of migraine and unclassified headache including one to two subjects (184, 201) identified abnormalities in relation to the brainstem, in keeping with the putative role of the brainstem in pain transmission pathways, including the descending modulation of pain (1, 32). Larger experimental studies including those by Gee and colleagues (n=277) (183) and Tortorella and colleagues (n=79) (174) (quality assessment ten and eight respectively, from maximum 12) also suggested that presence of brainstem demyelinating plaques might be associated with the occurrence of migraine. In contrast, Kister and colleagues (154) (n=204) (quality assessment 12) compared MS groups with and without migraine, and found no differences in the number or distribution of lesions (including brainstem) between the two groups.

8.2.2.1.4 Trigeminal Neuralgia and Trigeminal Autonomic Cephalalgias

Studies characterizing trigeminal neuralgia (TN) and trigeminal autonomic cephalalgias (TACs), in contrast, focused on abnormalities related to the trigeminal nucleus and nerve. Interestingly there appears to be overlap in radiological findings

between TN and TACs, though this observation may not be generalizable to patients without MS.

8.2.2.1.5 *Neuropathic extremity pain*

Neuropathic extremity pain of central origin (typically a chronic “burning” pain affecting the lower limbs (15)) is thought to be one of the most common pain syndromes in MS (5, 304). The included studies examined differing types of limb pain. The hypothesis that spinal lesions may be causative in limb or radicular pain has been examined in some studies.

In particular, in case reports or series, (173, 194, 202, 205) dorsal cord lesions in the thoracic and/or cervical cord have been linked to limb pain. Authors have suggested that demyelinating lesions may be linked to the occurrence of pain by directly disturbing sensory afferent pathways, or by disrupting descending inhibitory pathways (176) (205). Svendsen et al, however, in a study including spinal and brain MRI (n=25, quality assessment 9) found no association between site of demyelination and presence of chronic central neuropathic pain (176). Only one study (176) examined brain MRI imaging correlates of the presence of neuropathic limb pain.

8.2.2.1.6 *Location of lesions in the included studies*

Taking into account all identified studies, demyelinating lesions thought to account for pain syndromes were most commonly reported in the brainstem, and less commonly in the spinal cord. This may well, however, be linked to the observations above that the majority of studies investigated headache or facial pain syndromes.

Methods used for identification of culprit MS lesions also frequently relied on *a priori* anatomical hypotheses. This could in theory diminish the likelihood of identifying novel associations with a particular pain syndrome.

8.2.2.1.6.1 *Lesions affecting cortex, thalamus, or cortico-thalamic connectivity*

Limited studies specifically assessed the thalamus or its projections.

Notably, among the included experimental studies, Svendsen and colleagues (176) investigated any effects of MS lesions on cortico-thalamic projections. They found

no statistically significant difference in thalamic or thalamo-cortical projection lesion load in MS patients with or without pain.

Deppe and colleagues, using serial Diffusion Tensor Imaging (DTI imaging), studied a patient with central pain and abnormal somatosensory and thermal sensations on the right side of the body, and compared serial DTI data from this patient with imaging data from 100 healthy volunteers. The patient was part of a pilot for a clinical trial (188). The imaging technique and post-processing methods were well described. The authors suggest that the unilateral temporary increase of the fractional anisotropy found in the contralateral thalamus may have played a causative role in development of this pain syndrome, though the pain syndrome was relatively poorly described.

8.2.2.1.6.2 Normal-appearing tissue

Disease processes affecting normal-appearing tissues may be apparent when more advanced acquisition and/or analysis techniques are used. In the identified studies, any possible role of MS-related damage in normal-appearing tissue was not considered, with the exception of Deppe and colleagues' study, which used DTI imaging (188).

8.2.2.1.7 *Inferring causality: timing of MS lesion in comparison to occurrence of pain syndrome*

In many studies, the association of a demyelinating plaque with a specific pain syndrome was based on demonstration of the plaque's presence in a neuroanatomically plausible location at time of imaging for investigation of the pain syndrome. In some studies, the temporal association between the plaque, and occurrence of the pain syndrome, was further investigated by either carrying out serial imaging, or by using contrast administration. Evidence for temporal association of a plaque with pain syndrome could be relevant to investigation of possible causality.

8.2.3 Prospective experimental study: Clinical, behavioural and neuropsychological associations of neuropathic limb pain in adults with relapsing remitting multiple sclerosis

Study groups: demographics and medication

Groups of people with MS and neuropathic pain (“MS pain” group, n=31) and people with MS without pain (“MS control” group, n=16) were matched for age and gender, and furthermore balanced for disability, duration of MS disease and years of full-time education. All of the MS pain group, while meeting clinical definitions of established neuropathic limb pain, also met EFNS and IASP definitions of definite neuropathic pain (4, 217). Detailed phenotyping of the cohort is reported.

Accounting for potential effects of medications is complex (56), however the groups did not vary to a statistically significant degree in consumption of weak opiates, antidepressants (when all antidepressants were considered), tricyclic antidepressants specifically, baclofen, or MS disease modifying treatments. A higher proportion of the MS pain group were receiving gabapentinoid medications (55% vs 0%, $p=0.0001$).

Differences between MS pain and MS control groups

Statistically significant differences between the pain and control groups were found in a range of variables relevant to established descending pain modulatory pathways. These included scores for depression, anxiety, fatigue and catastrophising, as well as number of subjects endorsing symptoms consistent with major depression by the HADS instrument (157).

The MS pain group performed worse in tests of verbal and visual memory, but not in processing speed. The author is aware of only one previous study of cognition with respect to pain in MS, which reported differences in processing speed (53). The MS pain group performed worse in tests of cognitive flexibility, but not in other measures of executive functioning, including concept generation and inhibition. The author is not aware of any other tests of executive functioning, in particular cognitive flexibility, in relation to pain disorders related to MS. Given that processing speed is

reported as one of the most frequently affected cognitive domains in people with MS (48, 51, 306), the data do not suggest a global cognitive dysfunction.

Targeted quantitative sensory testing found higher rates of allodynia in the MS pain group, than the MS control group, consistent with deficient descending inhibitory systems. Distribution of symptoms drawn by subjects on body diagrams, and mapped at the group level using a novel tool, was however similar in the two groups.

Quality of life measures from the SF-36 were worse in the MS pain than MS control groups. Statistically significant differences were seen in five of eight subscales (four of four physical subscales, one of which measures pain, and one of four mental subscales).

8.2.3.1.1 *Subgroup analysis: MS pain and MS control groups, after exclusion of subjects receiving adjuvant analgesia*

Because of the potential confounding effect of medications on the above analysis, in particular gabapentinoids, an exploratory post-hoc subgroup analysis specifically excluded subjects who were receiving any adjuvant analgesic drug. This analysis supported the findings of the overall analysis (above). Results should be interpreted with particular caution given the small sample size and post-hoc nature of this analysis, however in most cases the direction of any difference seen between the groups, and the differences attaining the 5% statistical significance level, were confirmed in this exploratory analysis.

These results, interpreted with caution, would be consistent with group differences attributable to reasons other than medication administration.

Correlates of pain severity

Assessment of correlates of pain severity within the MS pain group demonstrated that scores for depression, fatigue, anxiety, and catastrophising were significantly associated with increased pain scores. These findings were broadly in keeping with findings on comparing the MS pain and MS control groups.

Cognitive differences seen between the MS pain and MS control groups were not however seen in the analysis of correlates of pain severity. No statistically significant

association of any measured cognitive variable with pain severity were found. No trend towards effect was observed.

Consistent with the differences in quality of life between groups with and without pain, all eight of eight quality of life subscales generated by the SF36 instrument were significantly associated with pain severity. These included three “mental subscale” measures which were not significantly associated with the presence of neuropathic pain (social functioning, emotional wellbeing and role limitation by emotional problems). Pain interference data from the BPI further suggested that self-reported pain levels were strongly associated with interference in all measured activities of daily life.

Interpretation

Taken together, these findings suggest that emotional and affective variables are strongly associated with both the presence and severity of pain. Cognitive variables (measures of memory, and importantly of set-shifting or cognitive flexibility) were associated with the presence, but not severity of pain.

Post-hoc exploratory analysis of a small group of subjects who are not receiving adjuvant analgesics, suggests that the differences observed between groups may not be solely attributable to medication administration. Such inferences should however be approached with caution.

8.2.4 Prospective experimental study: Structural imaging associations of neuropathic limb pain in adults with relapsing remitting multiple sclerosis

Comparison of groups with and without neuropathic pain

8.2.4.1.1 *Lesion volume and distribution*

Overall lesion volume was not significantly different on comparison of the pain and control groups. Some region of interest analyses, however, suggested trends in the data which should be interpreted with caution.

When analysis was restricted only to the brainstem, lesion volume was significantly higher in the pain group. On whole-brain analysis of lesion topography using permutation testing, only a single voxel adjacent to the right lateral ventricle was found to be more likely to be involved by T2 hyperintense lesions in the pain group than the control group. In a brainstem-restricted region of interest topographical analysis, T2 hyperintense lesions were found to be more common in the lower/lateral pons and right cerebellar peduncle, in the pain group.

8.2.4.1.2 *Voxel based morphometry*

When regional grey matter volumes were compared across the entire grey matter, no significant differences were found between pain and control groups at the 5% significance level. Separately, the statistical threshold of $p < 0.001$ (uncorrected for multiple comparisons) was used to explore trends in the data. Using the less stringent $p < 0.001$ threshold (uncorrected for multiple comparisons) (in keeping with previous studies (80, 288)), grey matter volume was found to be increased in the pain group in the left midbrain, and decreased in the pain group in right parahippocampal gyrus and mid-posterior cingulate, as well as the occipital cortex.

No evidence for alterations in volume of key structures thought to be relevant to the descending modulation of pain were found. (No differences were found at the 5% significance level on restricting analysis to a single pre-specified three-dimensional mask of DPMS structures (29, 33, 96)).

References

Correlates of pain severity

No statistically significant association of lesion volume (whole brain or brainstem), lesion distribution or regional grey matter volume distribution was found with pain severity.

Correlates of pain duration

Only regional grey matter volume distribution was assessed in relation to duration of pain, in keeping with previous studies (79, 87). Reduction in volume in the left anterior cingulate was found to correlate with pain duration, at an exploratory statistical threshold.

Other findings

8.2.4.1.3 *Measurement of ICV using automated methods*

ICV measurement using SPM was judged to be satisfactory in comparison with the gold-standard manual ICV estimation, and was used for subsequent analyses.

8.2.4.1.4 *Exploration of cross-sectional cervical cord area estimation*

Cervical cord cross-sectional area data from these brain imaging acquisitions were comparable to limited data previously published in the literature, and may support further exploration of this technique.

Interpretation of findings

The findings discussed above could be in keeping with a multifocal and mechanistically heterogeneous disruption in relevant supraspinal structures and pathways. There are, however, several points to consider.

8.2.4.1.5 *Lack of statistically significant correlates of the presence or severity of pain, within the described DPMS mask*

Region of interest analyses within this mask are reported, at the 5% significance level corrected for multiple comparisons. Results at the $p < 0.001$ level within this mask are not reported. It is interesting that, despite clinical evidence compatible with impaired descending pain inhibition, particularly including cortical/cognitive variables most often localised to the prefrontal cortices (Chapter Four, page 119) no grey matter volume abnormalities in this mask were found at the stated statistical threshold. These results do not support a hypothesis of significantly altered grey matter volume between the groups studied.

It is possible that the mask does not include relevant structures. The creation of the mask was however based on established literature (1, 33, 65, 87, 96) as described above.

The deliberate inclusion of cortical volumes which are relevant to cognitive flexibility as described elsewhere in this thesis (Chapter Four, page 119) will have increased the overall volume of the mask and the number of comparisons carried out within it. It is however unlikely that this effect will have led to subthreshold results. Please see below for further discussion.

8.2.4.1.6 *Statistical significance level of reported results*

The results reported are significant at the $p < 0.001$ level, uncorrected for multiple comparisons in space, across the whole brain. This should be viewed as an exploratory analysis. While this threshold is less stringent than the threshold of significance at the 5% level, after correction for multiple comparisons, VBM results at these significance levels are widely reported in neuroimaging studies (80, 288, 307), even where subjects with a chronic painful disease are compared with healthy controls (in contrast to the current study which employs a disease control group).

References

Small studies have occasionally reported findings at the 5% significance level uncorrected for multiple comparisons (214).

The lack of statistically significant findings at the 5% level, after correction for multiple comparisons is therefore not unexpected. This would be in keeping with findings of several recent large studies of cognitive and affective symptoms published by other groups, including the MAGNIMS collaboration (91-93) as well as with recently reported studies of pain phenotypes in subjects with dementia (288).

8.2.4.1.7 *Structures implicated by the above analysis*

8.2.4.1.7.1 Brainstem

The higher volume of lesions in the brainstem in the pain group is compatible with the overall study hypothesis of impaired descending inhibition of pain, mediated in part by a disconnection syndrome. The brainstem is a key location in the descending modulation of pain, specifically the periaqueductal grey matter and rostral ventromedial medulla.

No differential lesion distribution affecting regions core to the descending pain modulatory system such as the periaqueductal grey matter, and rostral ventromedial medulla was found. The regions found to be differentially involved in the pain group are however thought to be involved in projections to the cerebellum and elsewhere in the brainstem, and could be associated with relevant tracts within the human brainstem (273). It should also be considered that T2 visible hyperintense lesions only partially reflect the local and wider distribution of white matter abnormalities in MS (78, 92), and in that context the reported findings regarding T2 hyperintense lesion should not be overinterpreted in terms of precise anatomical localisation.

The finding of increased grey matter volume in the pain group (at the exploratory $p < 0.001$ level, uncorrected for multiple comparisons), in the mid brain abutting the periaqueductal grey and the trigeminothalamic nuclei echoes previous similar findings in the literature (87, 95, 97) which have previously been interpreted as suggesting a response to tonic nociceptive inputs (79, 87). It should be noted in relation to the possibility of facial/cranial pain syndromes (previously associated in some studies with similar findings (96, 97) that facial/cranial pain was relatively

rarely reported by our study subjects, and lateralised facial pain was only reported by three subjects (one of whom reported periorbital pain, and two lateral jaw pain – Chapter Four, page 119). A similar number of subjects with non-painful sensory disturbance reported lateralised head/facial sensory change. The lack of correlation between structural abnormalities in this area, and preponderance of cranial/facial pain syndromes may suggest that volume increase in this region is not specific to the presence of facial/cranial pain.

8.2.4.1.7.2 Posterior Cingulate Cortex

Decreased volumes in the posterior cingulate, in relation to healthy controls, have been found in studies of people with fibromyalgia, migraine, trigeminal neuropathic pain, temporomandibular joint dysfunction, and other types of headache including hypnic headache (79, 87). While these observations does not speak to the relevant function of these structures in the phenotype of chronic pain, they do lend support to the suggestion that these structures may be relevant to the experience of pain. In a recent VBM meta-analysis the posterior mid-cingulate was implicated as reproducibly reduced in volume in VBM studies of pain syndromes. An accompanying neuroinformatics analysis suggested that this structure (in addition to involvement in pain) has been involved in explicit memory and action execution (95). Explicit memory in particular may be relevant to the current study, given memory impairments demonstrated in the subjects with pain (Chapter Four page 119).

The posterior cingulate has been associated with a multitude of functions in functional imaging studies including executive functions, episodic memory retrieval, emotion and pain (308). Regarding pain, the posterior cingulate has been linked to central representation of various pain syndromes including rectal pain, phantom limb pain, dynamic mechanical allodynia in neuropathy, and irritable bowel syndrome (308). The mid-posterior cingulate has been reported to be involved in a variety of fMRI studies using noxious stimulation (both cutaneous and visceral) (309). It has been shown to be anatomically connected to structures such as medial temporal lobes, and ventromedial prefrontal cortex, and is a principal node of the default mode network of resting state brain activity (310).

References

The posterior cingulate furthermore has been shown to be affected by a range of neurological and psychiatric disorder including depression, schizophrenia and Alzheimer's disease. Interestingly, the PCC has been implicated in the focus of attention and arousal state, perhaps specifically in regulating the focus of attention (310). There is specific evidence of a role for the posterior cingulate in the regulation of pain by attentional distraction, in an fMRI study of distraction from painful stimuli by a cognitive task. The posterior cingulate (among other regions including midcingulate, hippocampus, cerebellum and insula) was found to be preferentially activated in association with the diminution of pain perception by attentional distraction (109). A role in the focus of attention/interoception could be particularly relevant to the current study.

8.2.4.1.7.3 Parahippocampal gyrus

In the same VBM meta-analysis described above (95), the right parahippocampal gyrus (as in the current study) was found to be commonly affected in pain studies. However, it was reported to be increased in volume on average in the assessed pain studies (95). A neuroinformatics analysis of the literature pointed to a role of the parahippocampal gyrus in symptoms of comorbid chronic pain, cognition and emotion. The authors hypothesised that the right parahippocampal gyrus might be particularly linked to modulation of pain, and pain sensitivity (95). Although this meta-analysis found that parahippocampal gyrus volume was, on average, increased in subjects with pain, multiple studies (as described above) have discussed an opposite difference (ie a decrease in volume in those with pain) (87) in keeping with the current study.

Considering the individual VBM studies discussed elsewhere in this thesis (80, 96, 97, 288), Younger and colleagues described an increase in right parahippocampal grey matter volume in relation to increasing duration of temporomandibular joint pain. The authors hypothesised that this could be related to compensatory adaptations to chronic pain (97). Fletcher and colleagues hypothesized that temporal lobe volume changes might be compatible with a role in contextualizing painful experiences, perhaps by linking to interoceptive experience or autobiographical memories (288).

Specific laterality of volumetric changes is repeatedly reported in imaging studies, as described above, where the right parahippocampal gyrus and related structures (as in the current study) have been implicated. The reason for any laterality is not well understood, and the authors mentioned above (80, 95, 288) neither identified any previous literature directly relevant to the laterality of these findings, nor made specific hypotheses in this regard. Possible explanations for laterality of volumetric findings (which could be explored with focussed experimental designs) could include differential structural and/or functional connectivity of the right and left parahippocampal gyri to relevant cortical/subcortical structures, differential susceptibility of the right and left parahippocampal gyri to atrophy or volume expansion, differential ability of imaging paradigms to correctly identify volumetric changes in these structures on the left and right, reporting bias, or type 1 statistical error (ie false positive results).

8.2.4.1.7.4 Occipital cortex

Although previous studies have identified changes in occipital cortex structure in pain disorders (214) there is not a well-recognised role of the occipital cortex in pain processing or sensation. This finding may simply reflect the use of a less stringent statistical threshold (ie type 1 statistical error) and is not further discussed here.

Comments on exploration of cross-sectional cervical cord data, from available brain imaging acquisitions

8.2.4.1.8 *Feasibility of techniques:*

The upper cervical spinal cord was found to be included in nearly all available images at both P2.5 and C2/3 level, with the exception of one subject where C2/3 level was not included. Manual and Automated CC-CSA estimation were both feasible, though manual estimation was considerably more time-consuming.

8.2.4.1.9 *Relationship between manual and automated estimation*

Scatterplots examining relationship between manual and automated CC-CSA estimates, at both P2.5 and C2/3 levels, confirmed that they were linearly related. Review of scatterplots in comparison with line of equality suggested that there was no clear trend for one method to over- or under-estimate CC-CSA in comparison to the other. Bland-Altman plots confirmed acceptable inter-method agreement.

References

Neither of the two studies described above (118, 120) (Papinutto and colleagues in abstract form) have described comparison of manual and automated CC-CSA estimation. Future work should compare the utility of proprietary software (such as Jim (121)) and open-source software (such as Spinal Cord Toolbox (266)), as there is a potential cost implication for researchers and funders. Other aspects of reliability such as inter- and intra- rater reliability are also currently unknown.

8.2.4.1.10 *Inter-subject variability*

Sources of inter-subject variability are not well understood in spinal cord imaging in multiple sclerosis. Demographic variables such as age and gender, have been reported to show significant relationships with cross sectional cord area, as well as white matter and grey matter segmentations (122). ICV as well as vertebral dimensions have also been reported to show strong relationships with cord cross-sectional area (118, 122).

The current study suggests that CC-CSA at both the P2.5 and C2/3 levels are strongly correlated, and that these in turn are correlated to intracranial volume (ICV), though P2.5 CC-CSA appears more strongly correlated with ICV. CC-CSA estimates at both C2/3 and P2.5 levels also tend to be higher in males than females, though this difference did not attain statistical significance at the 5% level. This is consistent with previous literature (122).

In the only study reporting correlations between cervical cord cross-sectional area and clinical measures, including EDSS, in measures derived from brain volumetric data, EDSS was only moderately correlated with CC-CSA, and only after addition of a healthy control group to the multiple sclerosis group (Spearman correlation P2.5 - 0.34, $p=0.015$, and at C2/3 -0.30, $p=0.032$). Separate data for MS patients only were not presented, though the authors stated that only once healthy control data was included, was there a significant correlation between EDSS and CC-CSA.

Although the current data suggest a weaker relationship with EDSS in the current study, a differing statistical measure has been used, no healthy controls are included in the current study, and because the current study used patients with RRMS (median EDSS 2.5) in comparison with a mixed cohort of 17 RRM, 15 SPMS and 5 PPMS

with median EDSS of 6.0 (118), both inter-subject CC-CSA variability and variability in EDSS might be expected to be lower in the current study. Separate pathological processes may also operate in subjects with relapsing-remitting, and progressive forms of the disease (28).

In a conference abstract, Papinutto and colleagues (120) describe that correlation coefficients for EDSS predicted by UCCA from MPRAGE were higher than in this study and Liu and colleagues' study: Spearman $r=-0.74$ $p=0.006$ and Pearson $r=-0.66$ in one centre, and in another and in the matched UCSF cohort: $r=-0.73$, $p=0.007$; Pearson $r=-0.64$. This difference might relate to the use of a specific spinal cord coil, differences in image analysis, lower sample size ($n=24$ total for the results described, as compared to 45 in the current study and 37 people with MS (and 13 healthy controls) in Liu and colleagues' study (118). Further analysis will be possible once this data has been peer-reviewed and is available for review.

8.2.4.1.11 *Differences between pain and control groups*

Data from the current study (Table 49: Comparison of CC-CSA in study participants with and without neuropathic limb pain) quantifies automated CC-CSA at two cervical cord levels, with and without correction for ICV, in groups with and without neuropathic pain. CC-CSA at the C2/3 level, but not at the P2.5 level, was found to be higher in subjects with pain, than those without.

Possible explanations could include 1) statistical artefact (analogous to Type I error); 2) presence of lesions at C2/3 level which are not readily visible on the current imaging protocol and which could contribute to cord expansion 3) confounding factors such as medication, the effects of which are unknown and 4) inflammation /oedema in the group with neuropathic pain. Increases in cord cross-sectional area have previously been reported in RRMS in comparison to healthy controls (311) and tentatively linked to inflammation or oedema.

8.2.4.1.12 *Wider applicability of method*

The current exploratory investigation has demonstrated that manual and automated CC-CSA estimates can be extracted from volumetric MPRAGE brain data in the current small study, and that manual and automated estimates are comparable in a

subset of 15 subjects. Associations between CC-CSA and other imaging and clinical variables are similar to those previously reported (118, 122), however the wider utility of this method remains unclear, and would benefit from comparison with application of similar methods to tailored spinal imaging acquisitions in the same patient group.

In conclusion, estimation of CC-CSA from volumetric brain data using Spinal Cord Toolbox may be feasible. Further analysis including inter- and intra-rater reliability is required. Relationships of CC-CSA derived from this method, and CC-CSA derived by gold standard methods including specific spinal MRI sequences also remain to be clarified. No firm conclusions regarding CC-CSA in groups with and without neuropathic pain can be drawn from this exploratory analysis.

8.2.5 Prospective experimental study: Functional connectivity of the descending pain modulatory system in adults with neuropathic limb pain associated with multiple sclerosis

Mean functional connectivity of rACC seed in subjects with and without pain

In this study group of people with relapsing remitting MS, both subjects with and without neuropathic pain demonstrated widespread functional connectivity between a rostral Anterior Cingulate Cortex (rACC) seed and a bilateral network of cortical structures including frontal, prefrontal, parietal (including sensory cortex) and cingulate cortex, thalamus, cerebellum and brainstem. Many of these structures have been repeatedly implicated in central processing and representation of pain, as well as in execution of neuropsychological tasks discussed and described in Chapter Four (page 119)(64).

Lower connectivity between rACC seed and PAG, in pain group

In a prespecified analysis, those without neuropathic limb pain manifested stronger functional connectivity between the rostral ACC and the periaqueductal grey matter, than those with pain ($p \leq 0.05$, corrected for multiple comparisons within PAG ROI). A spherical mask encompassing all subdivisions of the PAG (110) was used. Despite the relatively large PAG ROI used, this difference was observed only in the ventrolateral PAG, a region repeatedly implicated in endogenous descending pain modulatory networks (115, 312).

Higher connectivity between rACC seed and DLPFC, in pain group

In a further prespecified analysis, those without neuropathic limb pain manifested weaker functional connectivity between the rostral ACC seed and the dorsolateral prefrontal cortex, than those with pain ($p \leq 0.05$, corrected for multiple comparisons within a bilateral DLPFC ROI). This difference was found in the right middle frontal gyrus (116), and overlapping with DLPFC regions of interest previously specified in the functional MRI literature (110, 296).

Adjusting for T2 hyperintense lesion volume diminishes or eliminates differential connectivity

Although T2 hyperintense lesion volume was not significantly different between the groups with and without pain (Chapter 5), adjusting for the overall lesion volume diminished (but did not abolish) the statistically significant functional connectivity differences between rACC and PAG. Adjusting for overall lesion volume removed any statistically significant difference in functional connectivity across groups, between rACC and DLPFC.

Increasing T2 hyperintense lesion volume is associated with differential rACC functional connectivity at the whole brain level

Increasing T2 hyperintense lesion volume was positively correlated with functional connectivity of the rACC seed to a range of structures including (but not limited to) frontal/prefrontal structures implicated in executive function and the descending modulation of pain, as well as the occipital cortex. The significance of this finding is not clear.

Trends towards correlation of pain severity with rACC:DLPFC and rACC:PAG functional connectivity.

Subject-rated pain intensity at the time of imaging was not correlated with rACC:PAG functional connectivity at the 5% significance level after correction for multiple comparisons in space. However, using a less stringent exploratory statistical threshold (without correction for multiple comparisons within the PAG ROI) there was a trend to increased connectivity to the dorsal/dorsolateral PAG in association with increased pain severity ($p \leq 0.05$, without correction for multiple comparisons). Similarly pain intensity was negatively correlated with rACC:DLPFC functional connectivity at the left middle frontal gyrus, but at the statistical threshold of $p \leq 0.05$, without correction for multiple comparisons.

Anatomical specificity of differential connectivity

Using a midline occipital cortex seed as a “region of no interest” to examine the specificity of the above functional connectivity findings, all connectivity analyses were negative at the specified statistical thresholds.

Interpretation of findings

These findings may support the hypothesis of differential DPMS functional connectivity patterns (including dorsolateral prefrontal cortex, rostral anterior cingulate and PAG, but not a “control” occipital region of no interest) in subjects with and without neuropathic pain. T2 hyperintense lesion volume might contribute to these differential patterns of connectivity, perhaps by interrupting relevant white matter tracts.

Lower rACC:PAG connectivity in the pain condition may be compatible with the stated hypothesis of disrupted descending pain inhibitory pathways in the pain condition. The significance of higher rACC:DLPFC connectivity in the pain condition (given that cognitive flexibility and other cognitive variables are found to be impaired in the pain group, in comparison with the control group) is unclear, though a compensatory functional reorganization could be considered, and in explored further in future studies. Some potential limitations of the current study are considered below (Section 8.4).

8.2.6 Findings of prospective study in relation to overarching study hypothesis

Clinical and neuropsychological data

In comparison of the pain and control groups, the observed differences in emotional/affective variables (including depression, anxiety and catastrophising) as well as apparently selective impairment of cognitive variables (such as measures of memory and of cognitive flexibility, but not processing speed) are relevant to existing models of cognitive and emotional/affective contributions to the DPMS (29, 33, 56, 87).

Allodynia was more common in the pain group, and sensitivity to pinprick was higher (both at the 5% significance level, before correction for multiple comparisons). Both are consistent with either descending facilitation, or impaired descending inhibition, of pain.

Structural Imaging

Structural imaging findings on the whole suggested possible trends in lesion distribution and grey matter volume, between the groups, which were not significant at the 5% significance level, corrected for multiple comparisons, when whole-brain analysis was used.

The findings of increased brainstem lesion volume could be relevant to disruption of descending DPMS tracts through the spinal cord to the brainstem, though topographical analysis did not identify lesions differentially situated around the PAG or RVM.

Differences in grey matter volume between pain and control groups affected structures previously identified as implicated in structural imaging studies of chronic pain syndromes (95) (posterior cingulate, parahippocampal gyrus, pontine nuclei). All of these are implicated in the central processing of pain (as well as in other cognitive tasks including memory and executive function). However these structures are not thought to be key components of the DPMS. Similarly, no volume difference in known cognitive/modulatory structures (frontal/prefrontal cortex) in relation to the

presence of pain was found. Decreased anterior cingulate cortex volume was found to be associated with increasing pain duration.

These data suggest that WM lesion distribution in the brainstem may contribute to disruption of the DPMS in people with pain, but that key components of the DPMS are not differentially affected by grey matter volume loss (other than with increasing duration of pain).

Functional imaging

The findings of differential functional connectivity between key nodes of the DPMS (rostral ACC, DLPFC and PAG (1, 110)) in those with and without pain could support the overarching study hypothesis. The observation that differences in connectivity are diminished or removed by statistically adjusting for T2 lesion volume, along with the observation that rACC functional connectivity varies in proportion to T2 lesion volume, could be consistent with a role of T2 lesion volume in functional connectivity alterations, for instance by disrupting white matter tracts.

8.2.7 Participant experience of study

Overview

Thirty study participants took part in a pseudo-anonymised survey of participant experience, which was administered using a standardised survey instrument, with research participant input into design of the survey. The question format used was similar to that of previous studies (299) using a seven point scale for numerical feedback, and free text for comments.

Participation in feedback

Participation in feedback survey was relatively high (64% of study participants). Survey respondents and non-respondents were not significantly different in terms of age, or presence/otherwise of chronic pain at time of study participation. Those who participated in the feedback survey were, however less disabled on average ($p=0.04$). There was also a trend to higher numbers of female respondents ($p=0.054$). This could reflect easier access to the survey instrument among people with less disability, though efforts were made to make it available both online, and in paper versions. Alternatively, there may be differing levels of interest in participation, or differing

References

ability to take part due to, for example, competing priorities. Participation in research feedback has not been extensively studied, however one previous study of 252 people taking part in RCTs of treatment for depression found that respondents to an experience study were more likely to be female, and older (Tallon et al) (313). Any reasons for a gender imbalance in respondents is not clear.

Overall satisfaction with study participation

Overall satisfaction with involvement in the study was rated as high, as were most individual aspects of the study including aspects of clinical, neuropsychological and imaging assessment. Participants gave useful, informative, comments on particular aspects of the study which might help to inform the design of future studies.

Experience of particular parts of the study

8.2.7.1.1 *Information exchange*

The amount of information given in the current study, and the way in which it was presented, seemed to be viewed favourably by participants. The production of a study newsletter, while demanding in terms of workload to PF, was also viewed favourably and could be considered as a standard part of future studies.

Limited previous research has found that study participants preferred detailed information prior to study information, and also feedback of study results (314). Both of these themes are mirrored by feedback given in the current survey, although a minority of participants related that they were given too much, rather than too little information. The amount of information given can be individualised to some extent, though the use of standardised patient information sheets and consent forms (necessary for research ethics committee review and approval) requires a certain degree of standardisation, and there is no established optimal amount of information required for particular participant groups (314).

8.2.7.1.2 *Clinical and neuropsychological evaluation*

Questionnaires on pain, and other related issues including mood were included in the study. Some people might find these challenging, or feel that material was personal or intrusive. However, the content and delivery of the questionnaires and

References

neuropsychological assessment included in this study appear to be supported, overall, by the feedback obtained.

While some participants found the neuropsychological evaluation, in particular, challenging, overall ratings of satisfaction with both questionnaire and neuropsychological evaluations were high.

There is little existing consensus on which subjects participants are most likely to find distressing. A review of psychiatric studies has related that questionnaires exploring suicide, abuse, neglect, or bereavement have been found to be distressing to some participants in some studies (297). A minority in one study of 252 RCT participants with depression reported negative feedback about answering questionnaires in general (313). In that study 27 respondents (10.7%) made negative comments about questionnaires including covering personal issues, and difficulty understanding questions. In contrast, Schafer and colleagues (314) found that the majority of 763 European adults with depression or schizophrenia expressed willingness to take part in research using questionnaires (92%), interviews (84%) or psychological testing (83%) (314).

8.2.7.1.3 MRI and fMRI imaging

Participant feedback on MRI imaging was in general positive, for the current study. Some subjects did report that temperature of the scan room, noise levels, and comfort during MRI in general were a challenge. Overall, however, satisfaction with participation remained high, and contact with staff during MRI imaging was said to be good. This perhaps mitigated negative aspects of MRI imaging (315).

These themes reflect previous findings on participant experience of MRI imaging, which has been subject to more detailed research than other aspects of research participants' experience.

I am aware of one previous study (299) which recounted numerical ratings of comfort during MRI (specifically fMRI) procedures. The ratings obtained in that study were comparable to the current feedback, though the questions asked were slightly different. In that study data from 22 older patients undergoing research

neuroimaging, and 70 healthy volunteers undergoing research neuroimaging were presented. They were asked “how was the scanning procedure?” (answer via 7 point scale with the endpoints 1 “very comfortable” and 7 “very uncomfortable”). The healthy volunteers gave a median score of 3, and patients a median score of 2.5 (299).

Several studies of participants in clinical (rather than research) imaging have focussed on claustrophobia, which is viewed as a frequent reason for scan failure. In a clinical context, this may be justifiable, as the benefits of imaging usually outweigh the discomfort/risks to the participant. In a research study, however this is not necessarily the case, and study of participant experience should arguably be more detailed in a research context (299). Even seemingly minor aspects of the imaging experience – such as placement of the imaging department in the basement, as in this study – may adversely affect participant experience (315). The contrast between research and clinical imaging may be an artificial one, however, and limited data suggests that overall participant comfort is similar (299). It should also be highlighted, that people taking part in imaging research studies, are self-selected to tolerate imaging reasonably well (299).

Various aspects of research MRI imaging might be experienced as challenging by some participants (316), though existing knowledge is limited (297). In a study of 763 European adults with psychiatric problems, willingness to take part in research involving imaging was generally lower (75%) than willingness to take part in research using questionnaires or interviews (92% and 84% respectively) (314).

One systematic review of qualitative studies of patient experience (including 15 studies) mentioned several themes. Participants may dislike certain aspects of the MRI imaging process. They may need support during the scan, and available staff support may importantly impact on positive or negative experience (316). Specific challenging aspects of MRI imaging in general might include lying in a narrow space (claustrophobia), being in a strange environment, having to stay still, duration of imaging (research scans can take up to 120 minutes, and frequently 45-75 minutes (299)), concern about results (particularly for clinical imaging), noise (299), use of particular sequences (which may be particularly associated with varying noise levels

References

(299)), and perceived loss of control (315). Apprehension about results is probably less for participants in this study, but cannot be dismissed, particularly in the context of concern about ongoing disease activity and disease-modifying therapy.

8.2.7.1.4 *Noxious stimuli during fMRI*

Numerical ratings supplied by research participants suggest that they did find administration of noxious stimuli during fMRI moderately difficult. These ratings are however difficult to interpret, in the light of the phrasing of the questions used for these specific aspects of the study. Specifically, ratings of one to seven were used, as in the other parts of the survey. The other parts of the survey had however given anchor points of “not at all satisfied” and “completely satisfied”, whereas questions in this part of the survey gave anchor points of “not at all difficult” and “completely difficult” (in effect reversing the anchor point associated with positive, or negative, feedback. I am not aware of any previous research studies which have examined participant experience of noxious stimuli during fMRI.

8.2.7.1.5 *Motivation for research participation*

By far the most frequently-mentioned reason for study participation was altruism. Other motivators such as sense of interest, perceived benefit for the participant, and giving back to NHS/ARRNC staff were also mentioned. In this sample, only one participant reported that they were motivated to participate partly by the possibility of an MRI scan. This may provide some degree of reassurance with respect to any covert sense of coercion in this regard. Similarly, feelings of guilt were mentioned by only one participant.

These findings are similar to those previously reported, though published literature is small. In a multi-centre study of people with schizophrenia and depression (Schaefer et al)(314), the main motivation to take part in research was stated as altruism, though subjects also felt that they would like to help the medical profession. Some also felt that participation would give them access to better treatment. About a quarter considered financial incentives important (314), while only one participant mentioned this in the current study. In a further study of 252 people with depression who participated in trials of antidepressant drugs (Tallon et al)(313), most were glad they took part, and would consider participating in future research. In that study

people related their principal motivators for participation as altruism, doing something positive, feeling supported by researchers, and having time to talk. Some stated that they gained understanding of their disorder, and valued feedback on progress (313). Reasons for not participating in research have previously included concerns about privacy, data protection, causing psychological problems, time, or causing physical problems (314).

8.3 Relevance of this work to wider field

8.3.1 Systematic Review: Prevalence, associations and natural history of pain in multiple sclerosis

On comparison with previous estimates of pain prevalence, my analysis includes all studies used for prevalence estimates in a previous systematic review (5) with the exception of one (10) which was excluded in favour of a study examining an overlapping patient group. (7) By contrast, however, estimates of overall pain prevalence (63%; 95%CI 55% to 70%) (17 studies, 5319 subjects), (7, 11, 20-27, 44, 66, 142-146) and pain within the last month (62%; 95%CI 52% to 72%) (11 estimates, 4224 subjects)(11, 21-26, 44, 66, 145, 146) vary significantly from previous estimates (5) of 50% for point prevalence (three studies, 1872 subjects), (10, 23, 66) and 75% for pain within the last month (three studies, 854 subjects) (24, 44, 145).

This work therefore suggests that point prevalence of pain is rather higher than had previously been reported. Several factors including the prospective design of all included studies, the larger number of included prospective studies in comparison to a previous review (28 studies, 7101 subjects, in comparison to nine prospective studies, 3311 subjects), and use of weighted random-effects meta-analysis are likely to augment the accuracy of the current estimates.

This work is also the first to detail the prevalence of specific pain syndromes in MS (rather than overall pain prevalence) using a meta-analytic approach. Similarly this work was the first to quantify estimate heterogeneity, and to use a meta-regression approach to explore contributions to heterogeneity.

The published work arising from this chapter was recommended by the post-publication peer review “F1000 prime” website.

Enquiry about pain should remain a priority for clinicians treating all patients with MS. Investigation of the temporal profile of MS-related pain, and characteristics of patients at risk – using standardised study design - should also be clinical research priorities. Better understanding of the epidemiology of pain in MS could contribute to investigation of its aetiology, management, and potentially its prevention.

References

8.3.2 Systematic Review: Neuroimaging correlates of pain syndromes in multiple sclerosis

Neuroradiological studies of pain in MS are relatively low in number, and of variable design and quality. Some relatively rare pain syndromes (including Trigeminal Neuralgia) were the focus of a majority of studies. Other, more common, pain syndromes were less frequently studied. These findings may reflect the need for a shift in emphasis of neuroimaging studies in relation to pain in MS.

Significant methodological issues relating to study design, execution and reporting were also identified. Investigators using different study methodologies have reached differing conclusions regarding the neuroimaging correlates of specific pain syndromes in MS. Methodologically higher-quality studies were however less likely to report positive associations of lesion location to the presence of headache, or of chronic central neuropathic pain (154, 176).

Despite, therefore, the prevalence and impact of pain in MS, the insight into pain mechanisms currently afforded by neuroimaging studies remains limited.

The current evidence does support the hypothesis that focal demyelinating lesions are sometimes associated with the occurrence of specific pain syndromes in MS, in some cases. Study methodology has not, however, always been sufficient to further explore any association. Several studies have not found a clear association of lesion location with the occurrence of specific pain syndromes, and it is possible that MRI-visible lesion location does not explain a significant proportion of the burden of pain syndromes in MS. There is considerable opportunity to advance our mechanistic understanding of MS-associated pain, and thus its therapy, through future research.

8.3.3 Clinical, behavioural and neuropsychological associations of neuropathic limb pain in adults with relapsing remitting multiple sclerosis

The results described in this chapter suggest that, in the population described, emotional/affective factors are associated with both the presence and severity of neuropathic limb pain in relapsing-remitting multiple sclerosis. Cognitive factors (specifically memory and cognitive flexibility) are associated with the presence of neuropathic pain, but not severity of existing pain.

While the associations of affective and emotional variables with the presence and severity of pain in MS have been previously described, this study is one of the first to link cognitive factors to pain in MS, and to the descending modulation of pain specifically.

Interpretation of the difference between groups with and without neuropathic pain is limited by the higher usage of adjuvant analgesics in the pain group, however a post-hoc exploratory analysis limited only to those not receiving such drugs supports the conclusions of the wider analysis.

Both emotional/affective, and cognitive factors have been repeatedly linked to descending modulation of pain. The described results could be compatible with the study hypothesis of impaired pain modulation, in the population described. The differential associations of emotional/affective factors (including fatigue) and cognitive factors, with the presence and severity of pain, could be compatible with a model of dissociable mechanisms within an overall framework of the descending pain modulatory system. These findings are relatively less likely to demonstrate a “global” effect whereby cognitive and affective variables are always closely interlinked.

Such findings might be consistent with modulation of pain perception by both cognitive pathways and emotional/affective pathways. These pathways have previously been described as neuroanatomically and mechanistically distinct (31).

These results, if confirmed in future larger studies, could be relevant to understanding of pain modulatory mechanisms in MS, and could lead to further

investigation of how these systems might be impaired (for instance by focal demyelination, or by grey matter atrophy).

These results are among the first to explore the cognitive correlates of neuropathic limb pain in MS, and support future consideration of cognitive as well as emotional/affective and sensory aspects of pain in this disorder. From a treatment perspective, these findings could eventually be relevant to development and administration of targeted therapies (57, 260), including cognitive behavioural therapies. The finding of deficits in memory and cognitive flexibility in the pain group should also be borne in mind, in the design of any cognitive behavioural intervention.

8.3.4 Structural imaging associations of neuropathic limb pain in adults with relapsing remitting multiple sclerosis

The current data suggests that the described clinical and neuropsychological associations of the presence and severity of neuropathic limb pain in RRMS are in turn associated with trends in MRI measures of brain structures. Although these findings echo findings in people with chronic pain disorders outside the context of MS (95), few of these are in structures usually recognised as key to the descending modulation of pain.

Only one previous published study (214) has used computational techniques (voxel based morphometry) to assess grey matter volumes, and only in a subset of the included 23 patients. The current study is the first to report findings at a significance threshold more stringent than $p < 0.05$ uncorrected for multiple comparisons.

At an exploratory statistical threshold, the pain group manifests lower grey matter volumes in the posterior cingulate and right parahippocampal gyrus, as well as higher volumes in the left midbrain trigeminothalamic nucleus area, adjacent to the periaqueductal grey matter. Increasing duration of pain is also negatively associated with grey matter volume in the anterior cingulate cortex.

These findings, reported for the first time in MS, are consistent with findings in previous studies of pain syndromes outside MS. Associations of increasing pain duration with decreased grey matter volume may suggest a causative link between

the experience of pain and grey matter volumes, though further longitudinal studies would be needed to explore this hypothesis.

In a brainstem-restricted analysis, T2 hyperintense MS lesions are found to be more common in specific brainstem locations in the pain group, and may be relevant to a disconnection syndrome, reflecting the complex interaction of white matter and grey matter abnormalities in MS (78, 85). This study did not find, however, a regional grey matter volume difference between the groups in pre-specified structures usually recognised to be key to the descending modulation of pain including frontal and prefrontal cortices.

The potential relevance of posterior cingulate and parahippocampal gyrus in pain, memory and attentional roles, all of which are potentially relevant to the current study (Chapter Four page 119) are discussed above.

In addition, an early exploration of cervical cord cross-sectional area measurements (extracted from the current data using open source software) has been carried out. This exploratory analysis suggests that further exploration of the reproducibility, reliability and utility of this technique is warranted.

8.3.5 Functional connectivity of the descending pain modulatory system in adults with neuropathic limb pain associated with multiple sclerosis

This small cross-sectional seed-based resting state fMRI study provides early evidence that functional connectivity of the supraspinal descending pain modulatory system may be disrupted in people with neuropathic limb pain in relapsing remitting MS.

Only one previous study has used functional MRI to examine neuroimaging correlates of the presence of pain in MS (214). The current work is the first to focus specifically on neuropathic limb pain, to focus on relapsing remitting MS (rather than a mixed-phenotype group), and to employ a hypothesis-driven seed-based approach

References

to fMRI analysis. The current work is also the first to incorporate lesion volume in the analysis of resting state fMRI connectivity.

Functional connectivity of the dorsolateral prefrontal cortex, rostral anterior cingulate cortex and ventrolateral periaqueductal grey matter is implicated in the presence of pain. T2 hyperintense lesion volume may contribute to this differential connectivity. These findings are described for the first time in people with multiple sclerosis.

Trends towards differential functional connectivity in the above structures in relation to pain severity were also found. These did not however attain statistical significance at the predetermined threshold and are discussed briefly above.

8.4 Weaknesses of methodology

8.4.1 Systematic Review: Prevalence, associations and natural history of pain in multiple sclerosis

Inconsistent methodology even in relatively homogeneous studies

The meta-regression analysis in this study demonstrated few significant links between study design, or study group, factors and pain prevalence estimates. These findings seemingly contradict previous findings that pain is more common with, for example, increasing disability and disease duration,(7, 20-23) and could be in keeping with studies finding no relationship. (24-27) However, in my opinion, even within a selected group of included studies, underlying effects could be masked by inconsistent use of diagnostic, inclusion and exclusion criteria, (5) or by low number of available studies. It is possible that the apparent significant effect of pain timeframe in headache studies, but not in overall pain studies, could reflect more consistent use of diagnostic, (155, 156) inclusion and exclusion criteria in headache studies. Higher adjusted R^2 values for other variables in headache studies as compared to overall pain studies could be explained similarly. Significant correlates of pain may be unidentified, or inconsistently studied. Specifically, few studies quantified psychiatric or neuropsychological dysfunction.

Although relatively high quality studies were selected, some methodological concerns in included studies were identified. Investigator blinding, longitudinal follow up, and control groups were all infrequently used, and externally available validated instruments were infrequently used in overall pain studies specifically. Deficient blinding and use of varying diagnostic criteria may be most likely to influence prevalence estimates. Infrequent use of follow up and of control groups may principally affect characterisation of the natural history of pain in MS, and assessment of differences between MS and other populations. In addition, all included studies were carried out in North America or Europe, which could limit wider generalisation of findings.

References

This review had several limitations. Inclusion of a relatively low number of studies may have limited the power of our analyses, as discussed above. Pain severity, or quality of life have not been studied, and pain related solely to MS treatment was excluded. The findings therefore do not reflect these factors. A lack of control data precludes direct comparison of pain prevalence in MS groups with the wider population, or to other chronic neurological diseases, although chronic pain prevalence in the general population (Europe and Israel) has been estimated at around 19%. (224) Lastly, retrospective estimates of pain prevalence at disease milestones from included studies are discussed in comparison to our prospective data, though all available retrospective estimates were not included, as this was not the focus of our review.

8.4.2 Systematic Review: Neuroimaging correlates of pain syndromes in multiple sclerosis

Definition of multiple sclerosis

Included studies, as discussed above, include those which do not fully describe diagnostic criteria used in application of the diagnosis of MS. Therefore although all study authors described the inclusion of only subjects with MS, the possibility of alternative pathology (such as similar disorders, including clinically isolated syndromes of demyelination, or neuromyelitis optica) contributing to pain needs to be borne in mind. Although inclusion of subjects experiencing such disorders might diminish my ability to draw conclusions with respect to pain syndromes specifically in multiple sclerosis, there may also be some considerable overlap in terms of pathological mechanisms, and symptoms (including neuropathic pain). For instance, neuropathic pain is well described in early MS (25) and in neuromyelitis optica (252).

Limitation to English language studies

This review was limited to studies published in English. This may limit generalisability of the study to patients, or to healthcare systems, other than in predominantly English-speaking cultures.

References

Search strategies

The search strategy was designed to identify studies describing neuroimaging correlates of pain syndromes in multiple sclerosis. It is possible that it did not identify some studies which related neuroimaging features of pain syndromes, which were not, however, a primary focus of the study. Given that neuroimaging correlates of pain syndromes in MS are rarely studied, however, the number of studies of this type are likely to be low.

Authors were not contacted to identify unpublished work. In particular this could lead to an over-representation of studies identifying a link between identified MRI abnormalities, when compared to those not identifying any abnormality, in the available literature.

Quality assessment of studies

The described quality assessment tool was used as a framework for evaluation of the quality of individual studies. This tool is similar to that previously used by Campbell and colleagues in two separate studies of people with low back pain (167, 168). It is comparable to other tools used to assess observational studies including the National Institute of Health “Quality Assessment Tool for Observational Cohort and Cross-Sectional Studies” (317), and also takes into account comments on review and interpretation of methods in studies of pain, described by authors such as Hayden and colleagues in their study of prognostic reviews for low back pain (169).

While presented a “total score” is presented for each study, by simply summing the number of quality assessment items identified in each study, this should not be interpreted as an ordinal scale, but rather as a brief overview of quality assessment of each study, as a framework for further discussion.

8.4.3 Prospective Study: general comments

Issues of methodology for the prospective study in general are discussed, followed by separate discussions of the relevant experimental chapters.

References

Cross-sectional study design

This cross-sectional study can only report associations between measured data. Causality cannot be inferred. Even in the presence of causality, the direction of any causality (or presence of a further mediating factor affecting both variables) could not be inferred from the current study design.

A longitudinal study design might help to disentangle such relationships. This was not however feasible within the confines of the current study. Such a study design might furthermore be vulnerable to differential participant attrition over time.

Generalizability of data

8.4.3.1.1 *Recruitment source*

The current study subjects are drawn exclusively from a tertiary MS clinic in a single region of Scotland. All have consented to take part in a potentially demanding research study. It seems likely, therefore, that the current data are not fully representative of the wider population with relapsing remitting MS in the community.

Efforts are however made by the clinical team to ensure regular clinic review of all patients with RRMS in South East Scotland, in order to optimise provision of DMTs. This measure may act to improve comparability of the clinic population, from which this study population is drawn, to the wider MS population.

8.4.3.1.2 *Demographics*

Median age in the current sample was 42.5 in the MS control group and 41.0 in the group with pain. This is younger than the age at peak prevalence of multiple sclerosis in Scotland (56 years for women, 59 years for men (318)). This predominantly female sample (81.2% female in the control group, and 80.6% in the pain group), reflects the female preponderance of prevalent MS in Scotland, though estimates suggest that about 27% of prevalent cases are male in Scotland (318).

8.4.3.1.3 *Pain Severity*

Median average pain severity in the MS pain group was 5. Pain severity is comparable to several published studies which relate an average pain severity of

between 4.8 and 5.8 on a 0-10 scale (70;105;106), though some describe less severe pain (79).

8.4.3.1.4 *Anxiety and Depression*

Symptoms of anxiety measured using the HADS instrument (107) were appreciable in both control and pain groups (median score 5.5 and 9.0 respectively). Symptoms of depression were most marked in the pain group (median score 5.00, compared to 1.5 in the control group). The number of participants reaching a threshold score of eight for significant depression (11/47) and anxiety (26/47) was somewhat higher than reported by Korostil et al (108), who reporting elevated anxiety scores in 20% of the sample, and elevated depression scores in 10% of the sample. Korostil and colleagues however used a threshold score of 10, rather than 8.

8.4.4 Clinical, behavioural and neuropsychological associations of neuropathic limb pain in adults with relapsing remitting multiple sclerosis

Patient self-report measures

Where possible, the measures used in this study were specifically validated in studies of people with multiple sclerosis. If this was not possible, measures validated in studies of people with other neurological disease, or which are widely used in studies of people with MS, were used. Preference was also given to measures meeting guidelines for pain research studies - including published expert consensus, and patient survey (3;4). In addition to these criteria, however, study instruments were also selected to minimize burden on potential study participants.

This concern was balanced against theoretical priorities including ecological validity of specific instruments, and may have led to use of specific instruments which met the criteria detailed above, but where debate in the scientific literature remains regarding their relative merits as compared to other measures. For instance the SF-36 is regarded as a quality of life measure (5;6) however has sometimes been criticized for undue emphasis on functional abilities (319). To this extent the choice of instruments included in this study has been influenced by pragmatism.

Neuropsychology assessment

The BICAMS battery used in this study is widely used in MS, incorporates validated instruments, and is relatively short and easy to administer. Tests of executive function are not, however, standardised and while every effort has been made to include tests which are validated, or at least widely used, in comparable studies, there is no readily available acknowledged executive function battery for people with MS.

Measures of IQ were not available for the current study. Instruments such as the National Adult Reading Test (NART) (320) could be included to estimate IQ without completion of detailed testing such as the Wechsler Adult Intelligence Scale (55), which could be burdensome to participants. The NART instrument tests pronunciation of a standardised list of words, and was developed as an instrument to estimate pre-morbid cognitive functions in people with intellectual deterioration. It has been shown in some studies that there is an association between performance on

the NART, and on tests including those for verbal fluency. In this study years of full time education was used to provide an estimate of intellectual attainment, and an estimate of premorbid IQ has not been used. It remains possible, therefore, that some of the associations seen in the study could be related to premorbid IQ (although the lack of difference between the pain and control groups for years full time education, as well as gender, age, EDSS and disease duration might make this less likely).

The design of the current study, in that participants with known significant cognitive deficit are excluded, is also somewhat different to the original application of the NART in estimating premorbid IQ in those with established cognitive impairment.

Sensory testing

The quantitative sensory testing reported in this study was targeted to eliciting signs of central sensitization. It was based on existing QST protocols such as those suggested by the DFNS but is much shorter. Results reported here may therefore not be directly comparable with those reported in other studies using QST. Findings in a group of matched healthy volunteers, without pain or neurological disorders, and without recent use of analgesia are however reported. The findings reported are comparable to those from previous studies (15).

Study analysis

As described above, all data in this study was stripped of patient identifiable information, and was allocated a four-digit study ID number, generated at random by computer, before analysis. Where analysis was complex (as for the SF-36 instrument, for example), analysis was standardised by use of a computerised algorithm. PF was therefore blinded to the identity of the study participant and therefore their status as regards presence, or severity, of pain. Further attempts were made to mitigate effects of any unintentional unblinding by the use of computerised data marking where possible. A subsection of the neuropsychology data was marked by RM. This data was cross-checked by PF to ensure inter-rater reliability.

Multiple comparisons

As previously discussed, multiple comparisons between and within groups are made in this study. Results should therefore be interpreted with caution. For ease of

References

interpretation, 5% significance level is used throughout (except where specifically discussed). Statistical correction for multiple comparisons has used only the most straightforward version of the Bonferroni correction. The Bonferroni correction was applied to each “group” of tests (for instance psychological/affective morbidity, or executive function tests) that were viewed as distinct by the author, and are presented in a single table in the Results section. It is possible that other authors might disagree with the grouping of tests (and therefore application of Bonferroni correction) though the anticipated purpose of tests was specified pre-administration by the author and study team.

More complex procedures for multiple comparison correction such as False Discovery Rate (FDR) were not applied because of the grouping of tests by planned purpose (as described). Further techniques for dimensionality reduction (such as Principal Component Analysis) were not used in this thesis. While some measures applied could be seen to measure similar constructs (such as measures of severity of pain) the study was not designed to repeatedly measure similar constructs, and study variables were not in general directly comparable.

Measures of pain severity from the Brief Pain Inventory, for example were highly correlated with each other, but while the BPI is validated for measurement of non-malignant pain (221) and the Pain Severity Index is widely used (223), use of PCA components would remove any such advantage. Furthermore, pain measures from the BPI were only moderately correlated with the pain measure from the SF-36 which enquires about pain over a different time scale.

Effects of medication

No subject stopped their regular medication for the purpose of study participation. Medication regimes are in some cases fairly complex. Study participants were not matched for administration of medications. On comparison of the MS pain group, and MS control group, significantly more people with pain were receiving adjuvant analgesics. This difference was explained specifically by administration of gabapentinoid medications (gabapentin and pregabalin) but not other medications such as tricyclic antidepressants.

References

When designing the study, subjects were not asked to withhold medication. This is in keeping with a comparable recent study (214). This decision inevitably implied that analysis of the available data would be complicated by consideration of possible medication effects.

The first reason for this decision was that it was not considered ethical to ask subjects to withdraw medication.

Secondly it was considered that a request to stop medications might influence study recruitment, in ways that might be hard to estimate or account for (for instance perhaps only people with mild pain would agree to participate).

Thirdly, the optimal duration of a medication-free period to avoid affecting the described study instruments (including fMRI) is not fully established.

Fourthly, some subjects might surreptitiously take medication prior to study participation (for instance if pain was anticipated to be manageable, but in the event was not, and they felt that medication was required). This would make analysis particularly hard to interpret, particularly as any such effect would not be apparent at time of analysis, and could reasonably be expected to be non-randomly distributed among the study group. For instance, those with worse pain might be more likely to surreptitiously continue medication.

8.4.4.1.1 *Could Gabapentinoid medications account for the observed differences in clinical, behavioural and neuropsychological measures?*

There is limited evidence regarding the cognitive, behavioural and clinical effects of gabapentinoid medications (gabapentin and pregabalin). What evidence is available, is drawn from studies in subjects with MS as well as other neurological disorders, and uses both patient reported outcome measures, and objective measurements (for instance of cognitive processes). Patient reported and objective measures do not always support the same conclusions.

8.4.4.1.1.1 People with epilepsy: objective measures of cognitive processes

Leach and colleagues (321) assessed the cognitive effects of gabapentin on 21 people with poorly controlled epilepsy, in an older study. Participants were administered gabapentin as an adjunctive treatment in a double blind, dose ranging, placebo controlled, crossover study.

Neuropsychological testing was administered repeatedly at 4-weekly intervals. Testing included measures of decision time, movement time, a threshold detection test, forward digit span, forward visual span, paired association learning, and Stroop test (the latter commonly used as a measure of cognitive flexibility or set shifting (64)). Symptom and life satisfaction indices were also measured.

No psychomotor score showed a statistically significant difference between subjects receiving, or not receiving, gabapentin. No memory test showed a deficit in those receiving gabapentin relative to those not receiving it. No test of executive functions showed a statistically significant decrement associated with administration of gabapentin. In fact, those receiving high-dose gabapentin appeared to perform better on paired association learning test (though multiple comparisons were made, and the latter conclusion should be treated with caution).

8.4.4.1.1.2 People with epilepsy: subjective self-report of symptoms

In the same study (321), patient symptom scores for tiredness were higher when receiving gabapentin, but only on high-dose therapy of 2400mg/24hrs. Patient symptom scores for cognition and fatigue did not attain statistical significance, though there was a possible trend towards worse self-reported symptoms when receiving gabapentin.

More recently (2007), Marson and colleagues carried out a large multi-centre unblinded randomized controlled trial of carbamazepine, gabapentin, lamotrigine, oxcarbazepine and topiramate for treatment of localization related epilepsy (322). This study is part of the “Standard And New Antiepileptic Drug” (SANAD) study. Of 1721 patients recruited, 377 were randomized to gabapentin. Self-reported symptoms were measured. Cognitive side effects reported on Gabapentin were comparable to those with other medications and included tiredness/drowsiness (34%

of 377 subjects), depression (10%), memory problems (19%), confusion/difficulty thinking (15%) and sleep disturbance (4%).

8.4.4.1.1.3 People with multiple sclerosis: subjective self-report of symptoms

Gabapentin has been used for a variety of indications in people with multiple sclerosis. These indications include spasticity, neuropathic pain, painful tonic spasms, trigeminal neuralgia and acquired pendular nystagmus (323). Reported side-effects in trials for spasticity and for pain include drowsiness, sleepiness, dizziness, and gastrointestinal symptoms (323). These overlap with patient-reported symptoms detailed above (321, 322).

8.4.4.1.1.4 People with multiple sclerosis: objective measures of cognitive processes

Despite suggestions that gabapentinoid medications may be associated with cognitive and fatigue symptoms, there is little literature addressing objectively-assessed cognitive processes in pwMS receiving gabapentinoid medications.

Oken and colleagues (324) carried out a retrospective analysis using data from 70 subjects with relapsing-remitting and progressive MS (EDSS less than 6.0, ie mobile without aid) who had been included in a trial of a 6-month yoga and exercise program. Results of the trial were reported separately.

In their retrospective post-hoc analysis, they assessed objective measures of cognition in those receiving, and those not receiving, at least one drug with suspected CNS depressant effects. This category of drugs was specified as including SSRIs, antiepileptics (not necessarily administered for effect on seizures, and most commonly gabapentin), baclofen, benzodiazepines and others. They also measured patient-reported symptoms.

Assessments administered included measures of sleepiness and mood, PASAT, a measure of set-shifting (adapted from the Cambridge neuropsychological test automated battery), Stroop colour-word test, and computerized measures of attentional shifting, divided attention, and reaction time. Measures of fatigue were also acquired.

References

Importantly, 74% of their 70 subjects were taking at least one medication with suspected direct CNS-depressant action. These subjects took a mean of around two CNS-depressant medications. In comparison to those not receiving such medications, those receiving these medications were older (mean 50.3 vs mean 44.7 years), more disabled (mean EDSS 3.1 vs 2.4), and reported more depressive symptoms (CESD-10 score 10.3 vs 6.2). All of these differences were statistically significant at the 5% significance level.

The only cognitive test shown to vary significantly between the two groups was choice reaction time, which was longer in those receiving these medications. Performance on other tests including PASAT, set shifting and Stroop interference was not statistically significant between the two groups.

Several measures of self-reported fatigue were however statistically significantly different on comparison of the two groups, with the medicated group reporting higher levels of fatigue. Fatigue was found to correlate with reported depressive symptoms, and given the higher prevalence of depressive symptoms in the medicated group, this introduced an important confounder.

Important caveats in interpretation of this study include that the effects of individual drugs were not studied, and the retrospective nature of the study. It should also be noted that there were important differences in the groups of those receiving and not receiving drugs with a suspected CNS-depressant action, in terms of age, disability, and scores on a measure of depression. All of these could be considered potentially important confounders.

8.4.4.1.1.5 Summary: limited literature regarding possible effects of gabapentinoid medications

Taken together, there is limited available evidence describing the behavioural and cognitive effects of Gabapentin in people with multiple sclerosis, and with other disorders. Measures of patient-reported fatigue and other symptoms have been reported to be associated with administration of gabapentin. Objective measures of cognitive processes (including neuropsychological assessments aiming to described

set-shifting/cognitive flexibility) have not, as yet, supported deficits in these measures associated with administration of gabapentinoid medications.

8.4.5 Structural imaging associations of neuropathic limb pain in adults with relapsing remitting multiple sclerosis

Specifically with regard to structural neuroimaging, the following weaknesses are noted:

8.4.5.1.1 Measures of integrity of normal-appearing white matter are not included.

Such measures could include Diffusion Tensor Imaging (DTI) for example. At conception of this study, it was felt that acquisition of these sequences would add an unacceptable time burden for subjects, and preferred to include resting state fMRI in order to explore hypotheses around connectivity of the descending pain modulatory system (see chapter Six page 243).

8.4.5.1.2 *Tailored spinal cord imaging is not included in this study.*

This was felt to be potentially time-consuming for the subject. In addition, at time of conception of the study a relevant spinal coil was not available, and it was not possible to access neuroradiological reports of spinal imaging at 3 Tesla field strength. While this means that necessarily the current study does not report full neuroaxis imaging, the decision to concentrate on supraspinal imaging is in keeping with the study hypothesis of disrupted descending inhibition of pain, and allows detailed focus on relevant well-characterised supraspinal structures using established methods.

Exploration of the application of cervical cord cross-sectional area estimation techniques to this data has been described, in order to allow some estimation of upper spinal cord structure.

8.4.5.1.3 *White matter lesions are assessed by only one rater.*

It is not therefore possible to comment on inter-rater reliability of the approach detailed. I have however assessed intra-rater reliability (with a considerable time gap between the two ratings, which would serve to diminish recall of the original segmentation process).

All structural imaging was, in addition, reviewed by an experienced consultant neuroradiologist (Robin Sellar) who was blinded to patient pain status (with regards

to both presence and severity of pain), and commented specifically on lesion distribution. Maps of lesion distribution were created by PF, following specific training, and using these reports. This measure was designed to maximise sensitivity and specificity of lesion identification. In addition, a conservative approach to lesion identification was taken, if there was any doubt regarding misidentification of an artefact, perivascular space or similar.

8.4.5.1.4 *Potential effects of medications on brain structure*

There is limited published evidence concerning associations between medications administered to study participants, and measures of local brain volume.

8.4.5.1.4.1 Brain structure and function in opiate addiction, without pain

Upadhyay and colleagues (325) used structural MRI brain imaging along with resting state fMRI imaging to characterize structural and functional brain alterations that might be related to long-term prescription opioid use. They studied 10 individuals dependent on prescription opioids, and compared these with 10 healthy individuals who were matched for age, gender and handedness. The study design was cross-sectional. Notably, subjects were excluded if there was any known dependence on alcohol or on other drugs, if there was any comorbid psychiatric or neurological disease, or if there was any pre-existing medical condition. Specifically, subjects with pain were excluded. It was noted that two of the opioid-dependent group had initially started opiates because of pain, though this was not an ongoing issue.

The authors describe that, in this very small study, opioid-dependent subjects had a significant decrease in functional connectivity for insular, amygdalar and nucleus accumbens seeds. They also demonstrated decreased anisotropy in pathways relevant to the amygdala, and in internal and external capsules. Amygdala volume, along with white matter anisotropy, was found to be related to resting state functional connectivity of the amygdala.

Because this study specifically assessed subjects who were addicted to opiates, and excluded those with pain or with neurological or psychiatric disease, I do not think that it is directly relevant to the current thesis. It does however suggest a context for other studies discussed below.

References

8.4.5.1.4.2 Brain structure in subjects receiving opiates, with pre-existing chronic low back pain

Younger and colleagues (326) used structural MRI brain imaging to assess longitudinal changes in brain structure in subjects given opiates for chronic low back pain. 10 participants with chronic non-radicular low back pain were administered oral long-acting morphine for one month, and completed structural brain imaging before commencing morphine, at completion of the one-month course (when morphine was stopped) and thereafter. Opiate dose was titrated by clinicians according to patient response (within pre-specified limits) and ranged from 165mg to 3120mg over one month (mean 2170mg).

Separately, a similar imaging protocol was applied to 9 subjects with low back pain who were administered placebo. These subjects were however scanned on a different scanner and coil than the opiate patients.

Both groups reported a decrease in pain severity (calculated using a mean of the four pain severity items from the BPI (221)). In the opiate group, pain severity decreased from mean 4.5 to mean 2.6 at the end of the month. In the placebo group pain severity decreased from 5.3 to 3.9.

Right amygdala structures were found to decrease in volume in the pain group, on comparison between the pre- and post-opiate structural images. Amygdala volume decrease correlated with opiate dose. Right hippocampus, rostroventral pons, and orbitofrontal cortex were also found to decrease in volume, though these decreases did not correlate to opiate dose.

Grey matter volume increases were found in the right hypothalamus, left ACC, right posterior cingulate, right ventral caudal pons and left inferior frontal gyrus. These changes correlated with morphine dose. Further regions showed a volume increase that was not correlated to morphine dosage and these included bilateral mid-cingulate, ventral posterior cingulate and adjacent parietal lobe. Significant volume decreases were not seen in the “placebo” group. Volume decreases were maintained at further interval imaging (average time interval 4.7 months).

References

This small study demonstrates volumetric changes in a range of structures which appear to be related to administration of opiates for a period of one month, and are not seen with administration of placebo. Weakness of the study however include very small sample size, use of placebo group with data acquired on different scanner and with different coil (though noise characteristics were similar), lack of matching of the groups, lack of randomization, lack of comment on any other medications administered and lack of behavioural measures other than pain severity.

Lin and colleagues (with Younger as the senior author) (327) carried out a further study to address some of the methodological concerns mentioned above, and to assess reproducibility of the previously-reported results.

In this study, 21 subjects with chronic nonradicular low back pain were randomly assigned to receive placebo or morphine therapy. Morphine was titrated as previously described, and placebo was also “titrated”. Imaging assessments were carried out in the same way as described above, though opiate and placebo groups were imaged on the same scanner with the same coil (3 Tesla acquisition, 8 channel coil, 1.2 x 0.86 x 0.86 voxel resolution). In this study the groups did not differ significantly for age, or duration of pain.

Both opiate and placebo groups reported a decrease in pain severity across the duration of the study (opiate group reduction 1.52 on the pain severity index of the BPI, and placebo reduction 1.46). Reduction in pain severity was not statistically significantly different between the pain and placebo groups.

As previously described, study groups were assessed for any longitudinal change in grey matter volumes. In the placebo group, no grey matter volume changes were found. In the opiate group, reduced grey matter volume was found in the amygdala (bilateral), orbitofrontal cortex, and pre-supplementary motor areas. Increases in grey matter volume were found in the cingulate including mid-cingulate, dorsal anterior cingulate and ventral posterior cingulate. These locations overlapped with, though were not the same as, those previously reported by Younger and colleagues (326).

Strengths of this study (considered in relation to the above study in particular) include use of the same scanner and head coil, and re-testing of the previous experimental findings. Randomised allocation was used. Pain severity was not statistically different between the groups at study start. Weaknesses include that groups were not matched for age and gender, that other medications including analgesia were not recorded, that behavioural measures were not reported and that subjects' belief regarding taking opiates (ie whether they believed they were taking opiates, or not) were not reported. Method of randomisation was also not specified. Importantly, this study is also very small.

These studies, taken together, do suggest that opiate therapy may be associated with a change in volume of particular grey matter structures, in people with low back pain. Both increases and decreases in local grey matter volume were reported.

The current study excluded subjects who were receiving morphine or other WHO step III opiates. While these studies provide a useful context, and suggest that administration of medications may be linked to local brain volumes (by unknown mechanisms) it is not known whether the medications administered to the study participants might be associated with similar changes.

8.4.5.1.4.3 *Relevance to current study*

The current study excluded subjects who were receiving morphine or other WHO step III opiates. While the studies described above provide a useful context, and suggest that administration of medications may be linked to local brain volumes (by unknown mechanisms) it is not known whether the medications administered to the study participants might be associated with similar changes. I did not find any studies examining structural correlates of administration of gabapentinoid medications, measured using brain MRI.

8.4.5.1.5 *Subset of MS lesions analysed*

Only T2 hyperintense lesion distribution has been described in the current analysis. Some other studies have also added analyses of T1 hypointense lesions, thought to represent focal atrophy (78). This was not felt to be necessary for the current study design.

References

8.4.5.1.6 *Collinearity between pain duration and age*

Age and pain duration were found to be positively correlated and thus age was not included as a variable of no interest in the VBM analysis of correlates of pain duration. This is in keeping with previous reports (96). Therefore findings reported here could reflect the effect of age, rather than pain duration per se.

8.4.6 Functional connectivity of the descending pain modulatory system in adults with neuropathic limb pain associated with multiple sclerosis

Could these findings be an epiphenomenon related to altered grey matter volume?

Detailed structural analysis of the described cohort has been carried out, focussing on voxel based morphometry, and analysis of the volume and distribution of T2 hyperintense lesions (Chapter Five page 188). The pain group, relative to the control group, were found to have higher grey matter volumes in an area of the midbrain/pons (trigeminothalamic nucleus, overlapping with pontine reticular formation). This difference was significant at the relatively lenient threshold of $p < 0.001$, not corrected for multiple comparisons. The PAG mask used for functional MRI analysis lies outside this area, and thus while it is not possible to entirely rule out altered grey matter volumes within this ROI, even at a relatively relaxed statistical threshold of $p < 0.001$ uncorrected, no volumetric difference was found within this region of interest. Similarly, no volumetric difference was found in volumes of cortical pain modulatory structures including DLPFC and rACC, in the VBM analysis.

Could these findings be an artefact of CSF signal?

CSF signal is a well-recognised source of extraneous noise in resting state fMRI data (75, 110, 115). Shared CSF signal between regions might act to either reduce or increase apparent functional connectivity, depending on local CSF dynamics. The rostral ACC seed and PAG ROI used may both incorporate CSF (from the interhemispheric fissure and aqueduct of Sylvius respectively), and the voxel size available for fMRI data makes it difficult to fully define the border between CSF and other tissues, as discussed by other authors (115). The DLPFC seed used could also incorporate CSF signal. While CSF signal may influence the above-described findings, the following observations may make this less likely:

1.1.1.1.1 Efforts to remove CSF signal from resting state data

All subjects' resting state data was subject to ICA denoising (Preprocessing of resting state data) including removal of CSF signal, where identified. Furthermore all subjects' resting state data was analysed including a CSF seed timecourse as a

covariate of no interest. Both of these strategies are intended to diminish the contribution of CSF signal to the overall findings, and are applied at the single subject preprocessing stage.

1.1.1.1.1.1 Were these strategies successful?

Qualitative inspection of the functional connectivity of the rACC seed region in both pain and control groups (Figure 32) confirms that CSF regions' timecourses are not typically closely correlated with the timecourse of the rACC seed (after the above preprocessing). Functional connectivity is seen to be strongly centred on grey matter structures with sparing of CSF structures (for instance lateral ventricles).

1.1.1.1.2 Anatomical restriction of DLPFC mask to grey matter

For the DLPFC mask, analysis was restricted to regions most likely to include grey matter (as described) by thresholding using a publically available grey matter tissue probability map packaged with FSL.

1.1.1.1.3 Repeat of analyses using mid-occipital cortex "region of no interest"

Furthermore, analysis of an occipital "region of no interest" was included. This was deliberately centred over the interhemispheric fissure, in order to mimic placement of the rACC seed in this respect. No statistically significant findings described using PAG and DLPFC regions of interest were recapitulated on analysis of functional connectivity with the occipital region of interest.

Could these findings reflect head motion during image acquisition?

As discussed above, head motion is known to not only introduce noise into resting state fMRI models, but also to induce spurious correlations in resting BOLD timecourses due to shared variance, especially in structures which are closely anatomically related (101, 102). This is felt to be an unlikely explanation for the observed findings for the following reasons.

1.1.1.1.4 Preprocessing of resting state data with respect to head motion

A range of methods have been used to "clean" the resting state data of extraneous noise, including the inclusion of 6 head motion parameters in the GLM model, and Independent Components Analysis (ICA) denoising. These strategies are thought to

have complementary roles in addressing any contribution from “gradual” and “sudden” head motion (100, 102).

1.1.1.1.5 Exclusion of subjects moving more than one millimetre

Any subject moving more than 1mm during the entire run (before ICA denoising) was excluded from analysis. This threshold is more stringent than used in some comparable studies (for instance Yu and colleagues (115) report that subjects had no more than 3mm translational movement, and Mainero and colleagues did not describe any exclusion of high-movement subjects (103)). Mean head movement between the groups with and without pain (prior to preprocessing) was small, and did not vary to a statistically significant degree between the pain and control groups (Preprocessing of resting state data).

1.1.1.1.6 Role of “sudden” head movement in introducing shared variance in anatomically local, as opposed to distant, structures

Regarding spurious correlations induced by sudden head motion (102) specifically, the functional connectivity examined in this study is between anatomical structures which are relatively anatomically distant (anterior cingulate and PAG, and anterior cingulate and DLPFC). In statistical analyses of preprocessing approaches to short and long-range functional connectivity, sudden head movements have tended to reduce functional connectivity between comparably placed structures (such as prefrontal cortex and cerebellum), whereas preprocessing strategies intended to reduce the effects of head motion in the relevant statistical models have tended to increase functional connectivity between similar structures (101, 102). In other words, sensitivity to correlation in timecourses would be expected to be decreased, rather than increased, by sudden head movement over the anatomical space discussed. Sudden head movement is therefore a less likely explanation for the described functional connectivity patterns.

Could these findings reflect differential lesion topography?

In a whole-brain analysis, lesion topography was not found to preferentially involve any specific structure at a threshold of $p \leq 0.05$ corrected for multiple comparison (Chapter 5). Even at the less stringent statistical threshold of $p < 0.001$, uncorrected for multiple comparisons, only an increased likelihood of posterior periventricular

lesions in a single voxel was observed. This finding is unlikely to be directly relevant to disrupted integration of the DPMS.

1.1.1.1.7 Focal Periaqueductal Grey matter lesions: distribution of brainstem lesions

As reported in Chapter Five (page 188), brainstem lesion volume was higher in the group with pain, than those without pain. It therefore should be considered that alterations in local neurovascular dynamics related to focal demyelinating lesions (78) could contribute to the findings in this fMRI study. The observed anatomical distribution of brainstem lesions however makes this less likely.

In the contrast of pain group: control group, using a nonparametric permutation technique to assess lesion topography (distribution in three dimensions), T2 hyperintense lesions were found to be more likely in the cerebellar peduncle, and basal/lateral pons. None of these regions overlaps with the described PAG seed, making a direct contribution of focal demyelinating lesions less likely.

1.1.1.1.8 Differential lesion topography affecting tracts linking rACC with DLPFC, and rACC with PAG.

In a whole-brain analysis, no preponderance of lesions in regions anatomically linking these structures was found. Future work will include masks of white matter tracts linking these structures, identified from DTI studies (65, 111).

It is possible that the observed diminution of differential connectivity between these structures, when T2 hyperintense lesion volume is included in the statistical model as a covariate of no interest (page 260) may reflect an overall role of lesion volume in disrupted connectivity, relevant to the current study.

1.1.1.1.9 Functional connectivity of rACC seed varies according to overall lesion volume

The observed findings that rACC functional connectivity varies in correlation with overall T2 hyperintense lesion volume (page 259) may support a hypothesis that white matter lesion volume is associated with altered functional connectivity. The observed positive correlation of lesion volume with functional connectivity (and absence of negative correlation) could suggest compensatory functional reorganization as has been reported previously in fMRI studies of people with MS.

This finding is in common with some, but not all, of the published literature (108). This finding is not yet, however, fully explained. Other explanations, including the possibility of differential mean head movement or type of head movement in association with lesion volume, should be considered. Strategies as described above have however been adopted to minimise the contribution of head movement to the observed data.

Could functional connectivity findings reflect differences in subjects' medications?

As discussed in Chapter Four, the subjects included in the pain and control groups were matched for age and gender. Furthermore there was no statistically significant difference between these groups in disability, disease duration, years full time education, current use of strong opiates (no subject was using strong opiates), current use of weak opiates (dihydrocodeine), current use of baclofen, current use of MS disease modifying therapy, current use of any antidepressant medication, or current use of tricyclic antidepressants (Table 19).

The pain group, however, were more frequently current users of gabapentinoid medication (gabapentin and pregabalin). While the effects of these medications on functional connectivity within the DPMS are currently unknown, it is possible that administration of these medications may influence the reported findings.

8.4.6.1.1.1 Discussion of previously published data

There is limited available evidence of the effects of gabapentin or pregabalin on resting state functional connectivity. I am not aware of any evidence of the effects of gabapentin/pregabalin on resting state functional connectivity in adults with MS.

8.4.6.1.1.1.1 Effects of medications on fMRI measures: *pharmaco-fMRI*

Knowledge of the effects of medication on functional MRI measures is most often drawn from task fMRI experiments, in which reproducible patterns of activation or deactivation in response to a “task” or condition, are modulated by administration of a medication (75). So-called pharmaco-fMRI may allow investigation of drug effects at a network level, and to identify anatomical or functional correlates of CNS drug action (328).

References

While this field is in its infancy, the available literature is expanding. Actions investigated include those of SSRIs and similar drugs in depressive disorders, anti-epileptic drugs, and medication for attention deficit hyperactivity disorder. A variety of tasks, however, are used in different studies, and often it is challenging to identify what (apparent) effects may be specific to a drug or class of drugs, to compare or combine separate studies, and to make inferences on application of these observed effects in clinical drug development. Approaches to this problem are under development (328, 329).

8.4.6.1.1.1.2 fMRI examination of effects of pregabalin

Harris and colleagues (330) studied the effects of pregabalin in 17 patients with chronic pain related to fibromyalgia. They used proton magnetic resonance spectroscopy, task fMRI and resting state functional MRI in order to better delineate possible central effects of pregabalin. 14 participants underwent fMRI. There was no control group. A 3.0 Tesla scanner with eight-channel head coil and concurrent physiological monitoring was used.

Seed based connectivity analyses using seeds in the right insula were employed. The task fMRI paradigm employed pressure pain applied to the nail bed.

Subjects with greater pre-treatment pain levels were found to manifest greater connectivity between the insula and posterior cingulate, and insula and inferior parietal lobule. Connectivity of the insula to the IPL was correlated to reductions in pain when pregabalin was administered. In task fMRI analyses, restricted to regions of interest in the inferior parietal lobule and PCC, both the IPL and PCC showed greater deactivations in response to evoked pressure pain after pregabalin (330).

This small study, without a control group, which focussed on specific regions of interest (anterior and posterior insula, inferior parietal lobule and posterior cingulate) suggested that functional connectivity of these regions might be linked to both pain severity, and to efficacy of pregabalin. Further studies, in subjects with fibromyalgia and with other pain conditions would however be required to test the reproducibility and relevance of these findings.

8.4.6.1.1.1.3 fMRI examination of effects of gabapentin

Iannetti and colleagues (331) assessed the effects of gabapentin administration on brain activity in response to painful mechanical stimulation of normal skin, and also to capsaicin-induced secondary hyperalgesia. They studied twelve right-handed male normal volunteers using a double-blind placebo-controlled design. A single high dose of 1800mg gabapentin (administered orally) was used.

Gabapentin was found to reduce pain-related activations in the bilateral insular/operculoinsular cortex, whether or not central sensitization was present. During central sensitization only, gabapentin reduced activation in the brainstem, and suppressed stimulus-induced deactivations.

8.4.6.1.1.1.4 fMRI studies examining possible effects of gabapentinoid drugs: themes

The work discussed above explores responses in volunteers with fibromyalgia, or in healthy volunteers. I did not find any study examining effects either in MS, or in subjects with structurally abnormal brains. The limited available evidence may suggest modulation of central pain mechanisms by administration of high dose oral gabapentin. The implications for functional connectivity in subjects with pain conditions are not clear.

8.4.6.1.1.1.5 fMRI study of pain mechanisms in MS: relevance to potential role of gabapentinoid medications

In one study of resting state functional connectivity in people with MS, with and without pain (214), three of 12 subjects with pain, and two of 11 subjects without pain were receiving gabapentin or pregabalin. Because of small sample size, no separate analysis regarding associations of the administration of these medications could be carried out. This study did not specifically assess any potential role of medications, including gabapentinoid medications.

8.4.6.1.1.2 Discussion of data from the current study

A post-hoc subgroup analysis of clinical data comparing people with and without pain, but restricted only to those not receiving adjuvant analgesics (Chapter Four, page 119) examined cognitive and affective variables in this small subgroup (n=13 from control group, n=9 from pain group). Although this analysis should be viewed

References

as strictly exploratory, similar data trends were observed within this subgroup analysis, in comparison to the larger analysis including those receiving medication. This finding is not conclusive but may support a hypothesis that these findings are related to presence of pain, and not solely administration of medication.

The differential connectivity patterns between rACC:PAG, and rACC:DLPFC, do not reflect the same direction of association (the former is increased in the control condition, the latter increased in the pain condition). Any general effect of gabapentinoid medication in reducing, or increasing, functional connectivity within the DPMS would not therefore explain these findings. A more complex interaction of gabapentinoid medications with DPMS functional connectivity however remains possible.

Although a small number of subjects in the pain group ($n=3$, see Chapter Four, page 119) were receiving weak opiates, this difference did not attain statistical significance. It is not possible in the current study to rule out a role of weak opiates in disrupting pain connectivity in the DPMS, which is thought to rely at least partially on opioid signalling (110). Subjects receiving strong opiates were however excluded from this study.

Lastly, it is a common finding that subjects with neuropathic pain, are more likely to be receiving therapy for neuropathic pain, than those without (81, 115, 214). It could be argued that, in approaching subjects with neuropathic pain from a clinical perspective, the effects of medication comprise an intrinsic part of the experience and phenotype of people with established neuropathic pain. Thus an attempt, in a clinical population, to reliably separate the roles of medication from those of other factors, would be very challenging, if not impossible, and may limit the applicability of findings to a real-world clinical population.

Use of seed based analysis, and default mode network (DMN)

This chapter (as discussed) uses a seed-based approach to investigate functional connectivity relevant to the descending modulation of pain. The rostral ACC seed used in this study demonstrates connectivity with a range of cortical and subcortical structures (Figure 32: Mean functional connectivity of rostral ACC seed, separately

calculated in pain and control groups), some of which overlap to a degree with the well-established default mode network (289). This is not surprising, as this network is consistently described to include the prefrontal, anterior cingulate, posterior cingulate, inferior temporal gyrus and superior parietal region (289).

Activity of the DMN has been linked to the brain lying “at rest”, and is consistently found to be deactivated during performance of tasks. Other resting state networks including a network thought to be related to executive control and working memory have been found to involve the anterior cingulate. The latter network has been said to involved the frontopolar area, prefrontal cortex, dorsal ACC, and superior parietal cortex (289). These networks have been found to be relatively consistent across subjects and across studies, with the posterior components of the DMN demonstrating most consistency.

The use of a seed based analysis in this study allows investigation of specific hypotheses regarding the functional connectivity of the descending pain modulatory system. The design of the current study does not allow comment on broader network connectivity in the neuropathic pain state in comparison to the control group, and it is possible that structures other than those investigated are differentially functionally connected in those with, and without, neuropathic pain in MS.

8.4.7 Participant experience study

Timing of acquisition of data

A particular potential weakness of this study is that feedback was gathered at completion of the study, when passage of time might lead to recall bias. Previous comparable studies have posted out surveys a week after study completion (though time to response not stated) (298), or used interviews immediately after study completion (299, 315) . The data reported from this study could be more prone to recall bias, though it may also reflect established reflection on study participation, rather than immediate reaction (described in one study as pre-contemplative (315)). The relative significance of immediate or delayed appreciation of study participation is not established. In this context, it is interesting that the general substance of feedback provided was similar to previous studies (taking into account differing contexts and methodologies).

References

Possible bias in participation

The sample obtained was apparently biased towards less disabled participants, and perhaps towards females. This is in keeping with previous studies (313), and raises questions around generalisation of the results. The reasons for greater participation from less disabled participants might include physical barriers to accessing study materials, or completing a form online or in paper format. The reasons for any trend towards gender bias are not however clear.

Sample size

The sample described is small, which again suggests that conclusions drawn from the available data should be viewed with caution. Given the size of the available literature, however, these data may still be useful to those designing future studies, or assessing their design (such as through research ethics committees). In particular, I am not aware of any such studies examining the research experience of people with MS, nor of people undergoing noxious stimulation as part of a study protocol. The findings described are of course specific to this study (299).

Numerical outcomes

The use of a one to seven scale is not validated though this was similar to methodology used by Szameitat and colleagues (299). I did not examine the effect of demographic or other variables on overall satisfaction rating, because sample size was small, and because overall satisfaction rating was high, with little variability between subjects (Median 7, IQR 7 to 7). Previous studies have found some variability between participants of different genders (299).

Questions used in instrument

Questions were deliberately simple and kept in a very similar format, with no attempt at assessing internal validity, or test-retest validity. Some questions (for instance those around specific fMRI procedures, where the scale 1 to 7 referred to a degree of difficulty rather than a degree of satisfaction) might have misled participants, and might contribute to the broad spread of results seen (see **Error! Reference source not found.**). This procedure should be modified for future feedback surveys.

References

General

We also do not know to what extent the reports given reflect the experience of the study, and how much underlying personal and/or psychiatric factors. It is likely not possible to separate these factors, particularly taking into account the high prevalence of psychiatric comorbidity in the study sample. A control group (in this context, perhaps a group undergoing a clinic appointment but not research study assessment) has also not been included. Inference of causality must therefore be limited (297).

Previous studies in psychiatric patients have found that participants who are distressed by study participation, are more likely to have mental disorders or symptoms, or risk factors for these (such as adverse experience, neuroticism or low social support(297)). In the current study, however, despite high levels of psychiatric comorbidity, satisfaction with participation was high.

I have also not explicitly assessed subjects' coping mechanisms (315), nor the perceived impact of study participation per se (297) . Analysis of qualitative data is so far limited, and would benefit from a more formal approach.

8.5 Strengths of methodology

8.5.1 Systematic Review: Prevalence, associations and natural history of pain in multiple sclerosis

This review has used a variety of methodologies which may act to increase the utility of findings.

In particular

- Only prospective studies have been selected (in order to maximize comparability of included studies)
- Only studies recruiting subjects with definite MS have been selected.
- A thorough search strategy including a “forward” search of cited articles (in order to increase sensitivity) was employed.
- Foreign language articles were included.
 - Translators of foreign language papers were almost all from a clinical neurosciences background
- Formal statistical meta-analysis techniques were used to quantify and to explore potential sources of estimate heterogeneity.
- Complementary methods were used to examine the natural history of pain in MS, including searching for longitudinal studies, and also looking for cross-sectional studies at particular disease “milestones”

8.5.2 Systematic Review: Neuroimaging correlates of pain syndromes in multiple sclerosis

This review is the first to examine neuroimaging correlates of pain in multiple sclerosis.

References

A qualitative descriptive approach to the literature has been used, including describing and assessing the methodology of included studies, as well as describing and assessing their findings.

This review should therefore act as a useful point of reference for the development of future study methodology, including the study described in this thesis. In addition, the findings of the described studies are also useful in providing an early insight into any links between imaging correlates of the MS disease process, and the occurrence of pain syndromes.

8.5.3 Clinical, behavioural and neuropsychological associations of neuropathic limb pain in adults with relapsing remitting multiple sclerosis

Clinical phenotyping

This study describes data from a well-phenotyped group of people with definite relapsing remitting MS, and definite neuropathic limb pain. The latter is defined both clinically, and (post-hoc) by research criteria (4, 217).

Preference has been given to use of instruments which are validated in MS populations. Where this has not been possible, instruments which are validated in non-malignant pain studies, or widely used in studies of people with MS (to ensure comparability with existing literature) have been used (see Methods: 119).

Burden to participants

The study protocol described was designed in order to minimise burden to participants. In particular, where possible, duration of tests was minimised, and care was taken to ensure adequate breaks and refreshment. These measures should mitigate any effect of fatigue on the data gathered in the study, though such an effect cannot be ruled out (56).

The study was in fact well tolerated by subjects. This data is presented separately .

Study assessments

Assessment was carried out by PF (all instruments) or RM (neuropsychology instruments). Both have experience of research studies assessing people with neurological disorders, in particular MS. PF was trained in administration of neuropsychology instruments by RM, in order to ensure that the same methodology was used.

The study design could be further enhanced by increasing participant numbers, by longitudinal recruitment, by multi-centre design, and by addition of IQ estimation. These measures were not however felt to be possible within the confines of a PhD project.

References

8.5.4 Structural imaging associations of neuropathic limb pain in adults with relapsing remitting multiple sclerosis

Study design

Only one previously published study has examined grey matter volumes in relation to the presence of pain in multiple sclerosis (in a small subgroup of subjects, using a statistical threshold of 5% in order to report trends in the data) (214). No previous study has used lesion distribution mapping, and no previous study has examined the grey matter correlates of the severity or duration of pain. The included subjects are phenotyped in some detail (see Chapter Four, page 119).

Use of matched disease control group

In comparison to many other structural imaging studies of clinical pain syndromes (79, 87, 95), groups of people with relapsing remitting MS, with and without neuropathic limb pain have been compared. Healthy volunteers are not included. The current study therefore examines a contrast in relation to a specific symptom (neuropathic pain) and its associated variables (see chapter Four, page 119) within the context of a chronic neurological disease. This could be compared to many previous studies which have examined the contrast between healthy volunteers, and those with a chronic pain syndrome (without matching for the presence of chronic disease or other associated variables).

The pain and control groups are closely matched for age and gender. Furthermore the groups are balanced for a range of further variables (Table 19).

High resolution isotropic structural imaging

The use of high-resolution isotropic structural imaging meets subsequent recommendations for studies of structural imaging in MS (332) and allows higher resolution of relevant structures, including MS lesions which are typically of the size of millimetres-centimetres. A lesion filling approach was employed to diminish the impact of local MS lesions on grey matter segmentation in VBM.

References

Visual review of analysis data

Statistical analysis of imaging data can be complex, and is prone to error which can be promulgated throughout subsequent analysis stages (75). In this study, care was taken to check analysis steps visually wherever possible. This was particularly the case for registration, and brain extraction in the VBM pipeline (which required adaptation in a number of subjects).

8.5.5 Functional connectivity of the descending pain modulatory system in adults with neuropathic limb pain associated with multiple sclerosis

Clinical control group

In contrast with most published pain imaging literature (103, 110, 115) a control group with relapsing remitting multiple sclerosis (but without neuropathic limb pain) has been employed, rather than a control group of healthy controls. This study design therefore helps to minimise possible effects of chronic disease in general.

Previous studies of DPMS functional connectivity have focussed on subjects with macroscopically normal brains (such as healthy volunteers (110), and those with chronic pain conditions which are not associated with a macroscopically lesioned central nervous system – including chronic back pain and fibromyalgia (115, 333)). This study is among the first to study the DPMS in a central nervous system disease associated with overt CNS lesions.

Clinical phenotyping and structural imaging.

The subjects with and without neuropathic pain are carefully clinically phenotyped, as discussed in Chapter Four (page 119). In particular the matching of subjects in pain and control groups for age and gender, and balancing of groups for a variety of other variables, acts to reduce the number of factors which could contribute to the described findings. They have also undergone detailed structural imaging assessment as discussed in Chapter Five (page 188).

Previous studies

No published study has previously specifically investigated functional connectivity of the descending pain modulatory system in people with multiple sclerosis (166). One previous study has used a network connectivity approach (dual regression) to examine network connectivity in people with and without neuropathic pain in multiple sclerosis (214), and identified reduced connectivity of basal ganglia structures to the default mode network, in people with pain (in comparison to those without). Specific functional connectivity of the rACC seed to basal ganglia structures in this study has not been examined, as this was not a prespecified aim of the study. Seixas and colleagues did however recruit a group of people with mixed subtypes of MS, and the clinical phenotype of the patients described is not directly comparable to those described here.

Processing strategies to minimise contribution of extraneous signal/noise

As described in some detail, preprocessing strategies have been used which are designed to diminish the influence of head motion, CSF and white matter signal among other factors. These included the exclusion of subjects manifesting head movement of over one millimetre, ICA denoising of all individual data sets with particular emphasis on sudden head motion, and inclusion of WM and CSF regressors as covariates of no interest in the fMRI analysis models. In addition, a “control” region of interest analysis was carried out using a target region in the occipital lobe, to examine specificity of the reported findings.

Overall absolute head motion of included subjects was low (less than one millimetre in all cases, following exclusion of only two subjects).

Location and functional connectivity of seed and masks is comparable to previous literature

As described, masks were set up according to previously published literature (both with respect to the structures assessed, and the specific masks used (29, 33, 65, 110, 296), and findings demonstrate some consistency with previously published accounts. For instance mean connectivity of rACC seed is comparable to Margulies and colleagues’ findings (295), and the ventrolateral PAG is specifically identified as

References

implicated in differential connectivity between the pain and control groups, as in previous studies (113, 115), even though this specific location within the PAG was not prespecified in the analysis). The DLPFC mask used was based on previously published findings, but also on maximal mean connectivity of rACC to DLPFC (within a prespecified volume of the DLPFC defined by Wiech and colleagues (65)). Thus, this mask assesses a volume of the DLPFC which is found to be maximally functionally connected to the rACC in this study, and findings may not reflect functional connectivity within the whole DLPFC.

8.5.5.1.1 *PAG: findings in current study*

The finding in the current study of increased rACC:PAG connectivity centred on MNI coordinates $x = -4$, $y = -32$, $z = -12$ is consistent with the involvement of ventrolateral PAG (defined in Coulombe and colleagues' study as centring on MNI coordinated ± 3 , -32 , -12), which has repeatedly been implicated in endogenous descending modulation of pain. This finding is also consistent with the authors' finding of vLPAG:rostral ACC functional connectivity (113).

The trend in the current study of increased ACC:PAG functional connectivity with increasing pain severity, centred on MNI coordinates $x = -2$, $y = -28$, $z = -6$, could be consistent with involvement of the dorsolateral PAG or lateral PAG (reported by Coulombe and colleagues as centring on MNI coordinates ± 2 , -32 , -5 , and ± 4 , -31 , -8 respectively). Both are reported to show functional connectivity to frontal/prefrontal cortex, striatum and hippocampus (113). Ezra and colleagues' structural atlas suggests that this location is closely related to dorsolateral PAG, and may be involved in lateral or ventrolateral PAG (114).

8.5.6 Participant Experience Study

Novelty of data

Data of this type are not routinely reported in patient research studies of any type, and in particular not in studies of MS or of pain. The data presented suggest which parts of the research experience may usefully be improved for future studies, as well as suggesting which parts are well tolerated. The current work has shown that it is feasible to gather such data (298), and that both numerical and free-text feedback have given useful information, which is applicable to design of future studies.

References

Pseudo-anonymisation of data

The data gathered during this study was pseudo-anonymous. This might help to minimise any perceived pressure which could influence the feedback provided. The anonymity of feedback provided was emphasised at several points, though it should be noted that participants (voluntarily) provided their study ID number at completion of the survey. This was included in order to allow linkage to their study data, and was not used to link to person-identifiable data. Having said this, participants may have felt that the data could be used in this way.

Participant engagement in development and execution of future studies at the Anne Rowling Regenerative Neurology Clinic

A part of this sub-study I have also set up a group of people who have RRMS, and are willing to be contacted for advice/discussion on design of any future studies at, or allied to, ARRNC (n=21). Similarly, a group of people who have RRMS and are willing to be contacted for participation in any future studies has been set up (n=29).

8.6 How has the field changed during the course of this PhD?

Some recent developments in relation to the broad themes of each experimental chapter are summarised.

8.6.1 Prevalence, associations and natural history of pain in multiple sclerosis

Further studies published since the initial systematic review described in Chapter 2, are described in more detail at 2.3.7.

These studies include estimates of pain prevalence in countries other than those included in my original systematic review (17, 41). These estimates are comparable to the estimates of pain prevalence suggested by my systematic review, however they expand our knowledge of the prevalence of pain in MS worldwide.

Knowledge of longitudinal variation in pain related to MS has similarly been enhanced, with a further longitudinal study from Australia (17). A large NARCOMS study, while not using detailed confirmation of each included case, suggests general trends in pain prevalence over time, using a large database in North America (165). Pain related to MS relapse, however, remains very poorly understood (despite publication of one further study (160)). A significant opportunity remains to better understand how the incidence and prevalence of MS pain relates to neuroinflammatory episodes (and of course this knowledge might be relevant to study of any interface between immunomodulatory treatments, and MS-related pain).

8.6.2 Neuroimaging correlates of pain syndromes in multiple sclerosis

Published evidence

Since execution of the search strategies described, only a small number of further relevant studies have been published (212, 214). A published description of headache along with a diagnosis of MS (especially where the clinical presentation might overlap strongly with ADEM) (212) does not add significantly to the conclusions described previously.

Seixas and colleagues (214) used a range of techniques which have not previously been applied to the study of pain in MS, in a small case-control study of people with mixed subtypes of MS, with and without neuropathic pain. While a difference was reported in Default Mode Connectivity (5% significance level corrected for multiple comparisons, using Dual Regression), differences reported in Voxel Based Morphometry appeared to be at the 5% significance level without correction for multiple comparisons. In addition, the direction of association (for instance if increased volume in a structure was associated with presence, or absence, of pain) was unclear. This work does therefore expand the emphasis of the existing field, both in analysis techniques used and in the perspective of analysis (for instance a focus on cerebral connectivity rather than lesion location). Seixas and colleagues' findings, however, should in my view be viewed as posing interesting further questions in the field.

Approach to MRI imaging experiments

MRI studies are increasingly set up as large international collaborative studies (for instance, for pwMS, as part of the European MAGNIMS collaboration, (92, 93)). A further large international collaborative study is the Human Connectome Project (100), and other large epidemiological projects such as UK Biobank (334) are acquiring imaging data on a subset of participants. These studies are able to take advantage of optimised MRI imaging paradigms including resting state fMRI. These paradigms (in particular for UK Biobank) are optimised for older and/or more disabled patients in that there is a short duration of imaging, with (in the case of resting state acquisitions) short TR (Cathie Sudlow, personal communication).

Large collaborative studies, using a centralised approach to analysis, may have advantages including a standardised, blinded, centralised approach to expert analysis; large sample size (or increased ability to recruit in rare disorders); spread of cost among many centres and ability to investigate technical inter-centre differences. Disadvantages may include the complexity of organising such experiments as well as technical factors such as accounting for different scanners or scanner set-ups at different centres.

References

Approach to resting state fMRI analysis

Substantial work has been carried out on optimal denoising of resting state data, partly driven by the collaborative projects described above, in particular the Human Connectome Project (HCP). Multiple automated approaches to ICA denoising, or “scrubbing” (motion censoring) are now available (104, 335), though these may require training on a sample dataset before use. There is no single accepted best method for elimination of head motion artefact currently, though optimal protocols will be evolved for the HCP and other projects.

Comparably, the approach to segmenting white matter lesions in MS is not currently standardised, and many authors use a semi-automated approach, perhaps with in-house software (which limits reproducibility of data). Recent work such as BIANCA, an automated lesion segmentation program which will, in future, be packaged with FSL (open source), may partially standardise approaches to lesion segmentation, therefore maximising comparability between studies and centres (336).

8.7 Potential improvements in methodology

Potential improvements to the methodology of the described study are discussed below:

Longitudinal study design

A longitudinal study design would allow investigation of possible causality (or at the very least, temporal relationships) in the development of a pain phenotype. This would allow investigators to address specific questions such as those posed below (8.8). This study design was considered at time of inception of this study, and ruled it out for the following reasons:

- Limitation of resources within constraints of PhD project.
- Limitation of time within constraints of PhD project.
- Complexity of analysis with large volume of data (particularly for imaging outcomes).
- Strong possibility of differential loss to follow-up (attrition) in pain and control groups, which could bias results in ways which would be hard to predict.
- Potential for MS relapses occurring during follow-up would be high. Any requirement for freedom from relapses at follow up would therefore be challenging, and could lead to delays in imaging.

Neuropsychological testing

As discussed above, IQ could be regarded as a potential confounder in this study, and has not been measured. Formal measurement of IQ might be too time-consuming, and a surrogate marker of IQ such as NART (320) could be used. This study does however include a measure of years full-time education (which was not statistically different in comparing the pain and control groups).

Some of the suggested neuropsychological examinations were relatively time-consuming and, while interesting, were often scored at ceiling or close to ceiling, thus limiting utility in the analysis. These included the Hayling sentence completion task drawn from the ECAS screen (238) and the “elevator with distraction” test from the Test of Everyday Attention (241). Future similar studies could consider, for example, a more difficult version of the Hayling.

Quantitative Sensory Testing (QST)

While, as discussed, a full QST protocol was felt not to be optimal for this study (in terms of duration and participant acceptability), future examinations could consider the use of calibrated stimuli (including vonFrey stimuli) in place of NeuroTips which do not allow fully calibrated stimulation.

Pain tolerance could be measured using a test such as the Cold Pressor Test (337) in order to further investigate pain responses.

Structural imaging

In order to more fully investigate hypotheses around connectivity of the DPMS, use of Diffusion Tensor Imaging (DTI) would help to delineate structural connectivity, in tandem with the lesion probability mapping approach described. This multiparametric approach is increasingly frequently used to study structural and functional connectivity in tandem (93, 100, 107). The acquisition of DTI data was considered for this study, but DTI was not employed for the following reasons:

- Increased duration of imaging
 - This is potentially relevant to participant tolerance of imaging, as well as available time in-scanner.
- Complexity and timescales of analysis taking into account other imaging data acquired, within the constraints of a PhD project.
- Anecdotal evidence that DTI imaging might be associated with reduced subject tolerance of imaging (due to noise, and muscle twitching)

References

Resting state functional imaging

The data in the currently described study includes 105 volumes (TR 3 seconds, total imaging acquisition 5min 23 seconds). Because of signal equilibration, the first three volumes were discarded, leaving a remainder of 102 volumes. This relatively small amount of data might limit sensitivity of analyses in this study. Accelerated acquisitions using TR of approximately 0.4s, as in the Human Connectome Project (HCP) have been found to increase the sensitivity of detection of resting-state signal fluctuation by up to 60% (despite loss in signal level) (100). Use of accelerated acquisitions of this type might increase sensitivity to salient experimental findings, while maintaining an acceptable scanner time for subjects.

At time of setting up this study, use of a TR of 2 seconds (which was available at the time of imaging acquisition, and would have allowed around 150 volumes in the same timeframe) was considered. I did not use a TR of 2 seconds, because of the potential for combining data with a separate study which was ongoing at that time at Oxford University (214).

8.8 Selected possible future research questions

Epidemiology

The prevalence and associations of pain in MS have now been well investigated, and it seems that while further research studies may well be published, it is relatively unlikely that there will be major changes in the current understanding of the field, in the near future. Knowledge of the precise associations of the presence and severity of pain may be increased by future studies.

8.8.1.1.1 *Pain in MS relapse*

Pain associated with MS relapse is poorly understood and quantified. It is not known how often pain is associated with relapse, and, perhaps more importantly, its impact on patient function, recovery from relapse, and later progress is also unknown.

A study assessing the pain symptoms of patients in MS relapse (and their self-rated pain related impairments, using the BPI for example), and then again at a follow-up point, could help to assess the above factors. A particular difficulty in design of such a study is case ascertainment – in particular sensitivity and specificity (considering that patients may not always wish to attend healthcare providers with an MS relapse, or that a deterioration in MS symptoms may be due to other causes than relapse).

A grant application for a similar study was submitted to the MS Society. This was well received by pwMS reviewing the application, but was not funded.

Clinical correlates of the presence of pain in RRMS

8.8.1.1.2 *Does presence of pain predict poorer outcome?*

Neuropathic pain in MS is associated with a range of adverse outcomes including pain interference, depression, anxiety, catastrophising, fatigue, poor sleep and poor quality of life. Any of these might correlate with disability or functional impairment, which might increase over time.

Given the associations of pain with a range of adverse clinical and behavioural outcomes, longitudinal follow-up of people with MS with and without pain could assess any divergence of clinical trajectories in those with and without pain at baseline.

References

Objective assessment of physical activity (eg using accelerometers) could furthermore assess physical activity levels, which could be an important mediator of any associations of pain.

8.8.1.1.3 *Can behavioural/affective phenotype predict future pain development?*

In a comparable experiment, examining the associations of pain as predictors rather than consequences of pain, baseline measurements of straightforward clinical variables (fatigue, anxiety, depression, catastrophising) could be evaluated as risk factors for the future development of pain in subjects with early RRMS. An experiment might take the form of baseline measurement followed by follow-up at one year and analysis using a logistic regression model, or similar.

Are the current experimental findings reproducible in RRMS, as well as other MS phenotypes and other related disorders (eg NMO)?

Similar experiments to those described in this thesis could be carried out in people with other subtypes of MS, or similar analysis could be carried out in data already acquired. Studies in more disabled populations could be particularly vulnerable to movement artefacts.

Similarly, comparable experiments could be carried out in limb pain not associated with apparent structural abnormalities of the central nervous system. A typical experimental “model” would include fibromyalgia. If similar fMRI findings to those described in RRMS were found, this could suggest that applicability of MS as a model of the “lesioned” central nervous system, to diseases of the “non-lesioned” CNS, could be further explored.

Mechanistic significance of study findings

Given the cross-sectional nature of this study, it is not known whether the described findings relate to factors predisposing to pain, factors associated with pain persistence, or may be sequelae of pain.

8.8.1.1.4 *Do structural and functional imaging characteristics precede or follow the development of a neuropathic pain state?*

A longitudinal study beginning at first presentation with Clinically Isolated Syndrome or early RRMS could be used to assess whether any identified correlates

of the presence of pain are noted before, or after, the onset of pain symptoms. Any longitudinal study would be potentially vulnerable to differential attrition (ie those with more severe health problems, or more severe pain, might be less likely to attend follow-up).

8.8.1.1.5 *Does treatment of a neuropathic pain state modify or reverse the clinical, psychological and imaging characteristics detailed?*

This study could be conceived of as a longitudinal intervention study, and could take various forms for instance a cross-over, or placebo-controlled, design.

8.8.1.1.6 *Does detailed analysis of task fMRI support the proposed 'heirarchical' model of DPMS connectivity?*

Does task fMRI analysis using a model designed to assess possible causality (such as dynamic causal modelling, or structural equation modelling) support the hypothesis of aberrant top-down regulation of the DPMS by DLPFC?

During the study described above acquired “task” fMRI has been acquired using controlled painful stimuli to the lower limb, administered via a Medoc Pathway system during fMRI acquisition. While this data is reported in the Appendix, this data could be amenable to such analysis.

8.8.1.1.7 *Is there a genetic component to the specified findings?*

As part of my study (not described in this thesis) DNA has been gathered from all participants, and ethical permissions to use this in local or collaborative studies has been obtained. Data from this small sample may need to be combined with other studies. To my knowledge, only one study has assessed any possible genetic component to pain in MS to date (338).

Participant satisfaction with study involvement

8.8.1.1.8 *What factors determine subjects' satisfaction with research participation?*

A database of study participants engaging in studies locally could be linked to patient satisfaction scores gathered, for example, electronically at time of study participation. Large numbers of participants would be available and multiple regression models regarding type of study assessments (for instance, with respect to MRI - duration of MRI, inclusion of specific sequences and so on) could be entered

as explanatory variables. This work could be useful in informing design of future studies, and in justifying design of studies to research ethics committees.

For researchers designing future studies, this information may be useful in the design of studies, and to support applications to ethics committees. A systematic review of factors influencing participant experience of studies, perhaps focussing particularly on people with MS, would further help to inform design of future studies. Design of future studies specifically at ARRNC may be assisted by creation of the patient involvement groups described.

Participant experience of research studies

Discussion

Appendix: Scopus Search Terms

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Multiple Sclerosis	((("multiple sclerosis"[MeSH Terms] OR "multiple sclerosis/analysis"[MeSH Terms] OR "multiple sclerosis/anatomy and histology"[MeSH Terms] OR "multiple sclerosis/blood"[MeSH Terms] OR "multiple sclerosis/cerebrospinal fluid"[MeSH Terms] OR "multiple sclerosis/chemically induced"[MeSH Terms] OR "multiple sclerosis/chemistry"[MeSH Terms] OR "multiple sclerosis/classification"[MeSH Terms] OR "multiple sclerosis/complications"[MeSH Terms] OR "multiple sclerosis/congenital"[MeSH Terms] OR "multiple sclerosis/cytology"[MeSH Terms] OR "multiple sclerosis/diagnosis"[MeSH Terms] OR "multiple sclerosis/diet therapy"[MeSH Terms] OR "multiple sclerosis/drug therapy"[MeSH Terms] OR "multiple sclerosis/economics"[MeSH Terms] OR "multiple sclerosis/embryology"[MeSH Terms] OR "multiple sclerosis/enzymology"[MeSH Terms] OR "multiple sclerosis/epidemiology"[MeSH Terms] OR "multiple sclerosis/ethnology"[MeSH Terms] OR "multiple sclerosis/etiology"[MeSH Terms] OR "multiple sclerosis/genetics"[MeSH Terms] OR "multiple sclerosis/history"[MeSH Terms] OR "multiple sclerosis/immunology"[MeSH Terms] OR "multiple sclerosis/legislation and jurisprudence"[MeSH Terms] OR "multiple sclerosis/metabolism"[MeSH Terms] OR "multiple sclerosis/microbiology"[MeSH Terms] OR "multiple

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Appendix: Study newsletters

Overview

Newsletters were sent out to all study participants with MS throughout the duration of the study.

The aim of these was to keep participants informed, and in particular to make them aware of the use their research data was being put to.

Feedback from previous studies had suggested that study participants were often uncertain how their research data was being used, and appreciated this feedback.

All participants consented to receive this newsletter, at time of giving consent to participate in the study.

Newsletters were designed to be read by patients and/or relatives, rather than a scientific reader. A priority was to impart insight into study progress and findings, while avoiding too much detail, and maintaining brevity. One of the later newsletters was also used to remind study participants about the patient experience substudy. Feedback on the study newsletters from study participants was positive (Appendix: Participant Experience of the Study).

MS Pain Study newsletter, Christmas 2016



Thank you for your participation in the MS pain study. This is just a brief newsletter to say thanks for participating, and to update you on early progress in the study.

Have a great festive season!

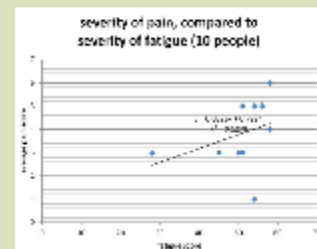
General Progress

So far 10 people are recruited into the study. We are aiming for a total of around 40. At the moment we're concentrating on scanning for people who are already in the study. We'll then move on to approaching more people about the study.

We're very much at the early stages, but results so far look interesting. I've included a few very preliminary pictures. (Of course these are just for interest, and aren't final results).

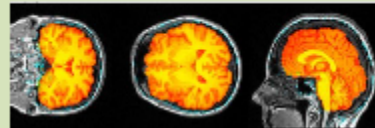
Questionnaires and other assessments.

The severity of someone's pain can be analysed, and compared to other findings. In the longer term we will link these analyses to MRI results. This graph shows a preliminary basic comparison.



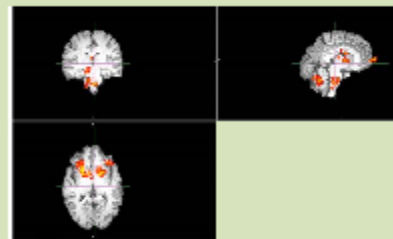
Structural MRI brain scans

These are some MRI sequences from the study, tested on a volunteer without MS. In people with MS they demonstrate some of the effects of MS on the brain.



Functional MRI brain scans

These 'functional' brain images are of a volunteer without MS, who is undergoing a mild painful stimulus. The yellow/red areas suggest which parts of the brain are involved in sensing or processing pain. We are investigating whether these areas are different in people with MS, who experience different levels of pain.



Future

We should finish MRI scans for the people already in the study reasonably soon. We'll then move on to recruiting more people, and further analysing the results.

Thanks again for your help in making this study a success.

Yours,

Peter Foley

On behalf of the MS pain study team

email: peterfoley@nhs.net phone: 0131 465 9511

MS Pain Study newsletter, Summer 2014



Thank you for volunteering to take part in the MS pain study. This is just a brief newsletter to say thanks for participating, and to update you on progress in the study. I'll send out a newsletter twice a year until late 2015 to keep you updated.

General Progress

So far 18 people have kindly taken part in the study. We are aiming for 30-40 altogether. Referrals for the study continue to come in from the MS team and other teams.

Information about the study online

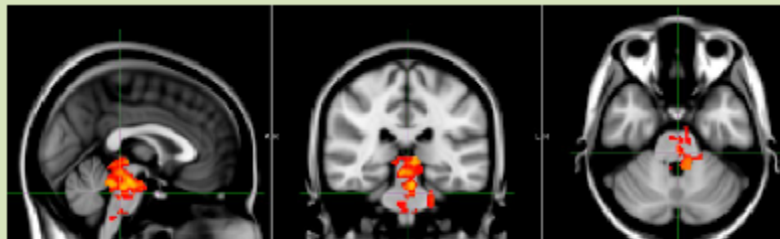
There is a brief piece about the study on the Anne Rowling Regenerative Neurology Clinic website at <http://annerowlingclinic.com/news/ms-pain-research-update>.

Scientific papers

The work you have been involved in hasn't been published in a scientific journal yet – as it is still ongoing. We have however published work looking at how common pain is in MS, what types of pain people get, and when they might be more likely to get pain (there is a link to this paper through the website above). We have also recently published work examining what is known so far about scan appearances in people with MS who have pain.

Optimising ways of analysing brain function in a "difficult" part of the brain

We have been looking at analysing the functional MRI scans (when you were given heat in the MRI scanner) and in particular investigating how to best see changes in the brainstem. The brainstem is a small part of the brain which can be difficult to "see" reliably on scans, but is crucial in pain processing. This preliminary work will be presented as a scientific poster at a British MRI research meeting in September (<http://ismrmannualmeeting.wordpress.com/>). The initial pictures below show response to pain specifically in the brainstem (shown as red/yellow), averaged across 8 of the people who have already taken part in the study.



Future plans

We aim to keep recruiting people into the study for the rest of 2014 at least, with the plan being however to focus more on analysing the data in 2015. The study will finish in late 2015.

Thank you again for your help in making the study a success

Peter Foley

On behalf of the MS pain study team

email: peterfoley@nhs.net phone: 0131 465 9511

MS Pain Study newsletter, February 2015



Thank you for volunteering to take part in the MS pain study. This is just a brief newsletter to say thanks for participating, and to update you on progress in the study.

General Progress

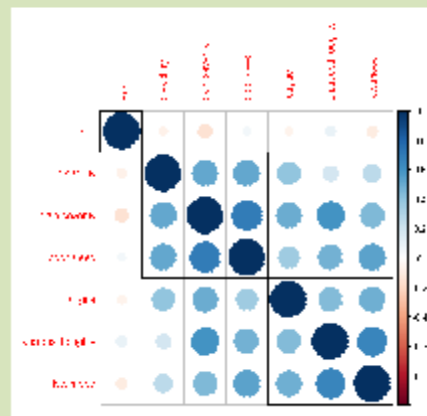
So far 31 people have kindly taken part in the study. We aimed to include up to 35 people with pain problems, so this is good progress. Here are some short updates:

Where do people in the study have pain?



- It's useful to work out where people notice pain, and to be able to show this quickly and effectively
- Each person in the study completed a diagram of where they have pain. We have combined these using a computer programme to create a single coloured picture (shown on the left here).
- This picture shows where people in the study experienced pain, with the darkest orange being the parts of the body where people most often noticed pain.
- This should be a useful tool for communicating results of the study.

What symptoms are linked to how bad the pain is?



- It seems as though several of the issues people report may be linked together in a complex way. This is a very early example of trying to investigate these possible relationships.
- You can see the possible strength of links between two symptoms, on average for all people in the study.
- Look for the name of one symptom on the left, then imagine a line running to the right from there
- Look for the name of the other symptom on the top row, then imagine a line running down from there.
- Where the lines meet, there will be a coloured circle
- The strength of the colour suggests the strength of any possible relationship – the darker the colour (and the bigger the circle), the more closely related.
- Please bear in mind that these results aren't definite!

Plans for feedback on how you found the study

We are planning, and developing, a very short questionnaire to ask your opinions on how you found the study. We plan to be in touch about this in the future.

Future plans

We are considering expanding the study to also include people who do not have pain, so that people with and without pain can be compared. The study will finish in late 2015. Thank you again for your help in making the study a success.

Peter Foley

On behalf of the MS pain study team

email: peterfoley@nhs.net phone: 0131 465 9511

MS Pain Study newsletter, May 2015

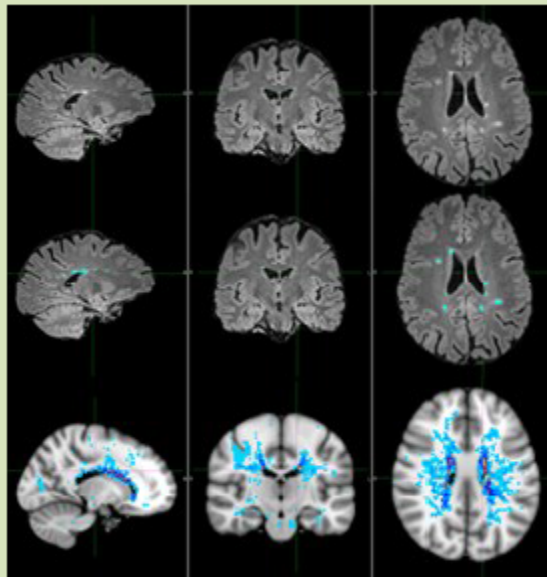
Thank you for volunteering to take part in the MS pain study. This is just a brief newsletter to say thanks for participating, and to update you on progress in the study.

General Progress

Since the last newsletter, the main news is that we've started to recruit people who have MS, but do not have pain, as a comparison group.

Here is a brief update on some other progress. We would be very grateful for your thoughts about plans for getting your feedback on the study (see below)

Where in the brain do people have MS lesions?



- We are trying to work out if the location of someone's MS lesions might affect their pain.
- MS lesions in the pain processing areas, or sensation areas, might be important.
- Each person's scans shows where they have MS lesions (top picture, the MS lesions are white/grey blobs)
- We can then identify these for each person (middle picture, MS lesions are now coloured in blue).
- We can then use these to work out where, on average, people are likely to have MS lesions (bottom picture, this shows an average for the whole group, with the more intense colours showing where MS lesions are most likely)

Plans for feedback on how you found taking part in the study

- We'd like to ask people who took part in the MS pain study about their experiences in the study. This will be useful in better understanding the experience of taking part in this study, and also for designing future studies.
- We are planning to use a very short survey.
- We have already started to design a survey, but would be interested in including areas which you might feel are particularly important.
- Can you help us to decide what questions to ask? At the moment, we are not asking for your opinions on the MS Pain study, but are interested in what areas you feel we should ask about.
- Please write any suggestions over the page, and post this back to the Rowling Clinic using the paid envelope provided. You can also email peterfoley@nhs.net.

Thank you for your time. We'll send out information on the feedback survey soon, once we have had time to gather any suggestions you might have.

Peter Foley, On behalf of the MS pain study team

email: peterfoley@nhs.net phone: 0131 465 9511

MS Pain Study newsletter, October 2015

Thank you for volunteering to take part in the MS pain study. This is just a brief newsletter to say thanks for participating, and to update you on progress in the study.

General Progress

We have now finished recruiting people into the study, and I am soon going back to regular ward and clinic work. Overall we had 31 people with MS and pain, and 16 people with MS but without pain who took part in the study. These numbers are quite good for a study of this type, and I'm very grateful to everyone who participated.

Information available from the study:

We have detailed information available from everyone who took part, and this includes questionnaires, psychology testing, examination findings, and MRI scans looking at brain structure as well as function.

The challenge is tying everything together!

Findings so far:

At the moment everything is being analysed. So far it looks as though there may be some interesting differences between people who have pain, and those who don't. Some of these differences seem to be seen on the questionnaires, and some on the scans.

I can't say too much at the moment though, as everything is being analysed, and it will take a while (and also review of the findings by others) until I can be more definite. I hope to get all this work published in a scientific journal. I will be in touch to let you know if/when this happens.

Feedback questionnaire

So far twenty nine people have kindly filled out the anonymous feedback questionnaire. Thanks very much for this! There are some interesting findings, both in the "ratings" numbers which people have given, and also in the comments people have made. If you would like to fill out the questionnaire but haven't yet been able to, you can still do so.

- This survey is online at <https://edinburgh.onlinesurveys.ac.uk/participant-feedback-sensation-and-pain-in-ms>
- Your password is: pathway
- Your 4-digit study number is:
- If you would like a paper copy, please phone 0131 465 9500, or email peterfoley@nhs.net
- Please do not include your name, date of birth or address when you fill out the survey.

Thanks again for your help.

This might be the last of the semi-regular newsletters. I will be in touch, though, to let you know of any publications, or any other developments.

Yours

Peter Foley, On behalf of the MS pain study team

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Appendix: Exploration of BOLD response to extrinsic thermal noxious stimulation: task fMRI

8.9 Introduction

In order to supplement data and methods reported elsewhere in this thesis, a task-based functional MRI paradigm was employed, in the same scan session as the resting state fMRI described previously.

In order to directly investigate the BOLD response to pain, a noxious stimulus was used. Because the perception of a stimulus was expected to vary across subjects, a thermal stimulus was employed. A thermal stimulus was used because this allowed variation of temperature, titrated against subject report of pain severity. The temperatures of thermal stimuli were calibrated individually against pain rating for each subject. In addition, the noxious thermal stimulation was judged to be qualitatively comparable to the burning pain reported by many patients with neuropathic limb pain in MS

8.10 Methods

8.10.1 Subject assessment

Immediately prior to imaging, each research subject was assessed in the mock scan room, adjacent to the 3T Siemens scan room, at the Clinical Research Imaging Centre, University of Edinburgh.

8.10.2 Thermal stimulation

An MRI-compatible Pathway device was used to enable thermal stimulation during the scan session as well as prior to the session (Medoc systems, Israel)(339).

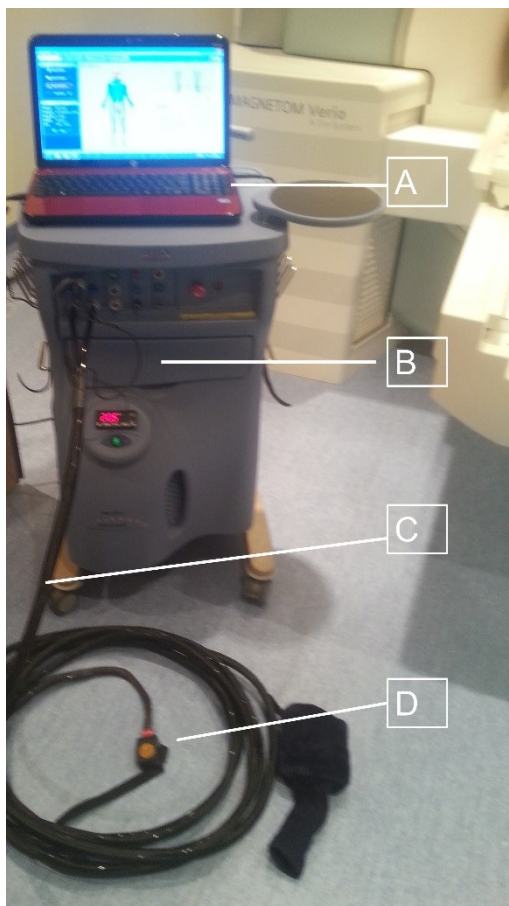
Set-up and installation of Pathway device

Set-up and usage of the device was discussed with local physicists (especially Neil Roberts, Scott Semple and Gill MacNaught, as well as researcher Jonathan Murnane) and with researchers from other centres (Irene Tracey, Oxford and Jon Brooks, Bristol). Jon Brooks assisted directly in setup of the device.

The Pathway device is shown below. Please note:

- Windows laptop [A] running proprietary software which controls the temperature administered by the thermode.
- The Pathway base unit [B] which generates the appropriate temperature for the ceramic thermode
- MRI-safe cabling [C] encompassing electronics as well as coolant circulating to the thermode (10 metres).
- The Ceramic Thermode [D] (ceramic disc enclosed within black box) including Velcro strapping

Figure 46: Pathway device set up in mock MRI room, Clinical Research Imaging Centre



Note: use of wave guide to allow connection to control room

During scan session, the Pathway device needed to be attached to the command module (see image above) from the scan room, to the control room. Because the device could not be left in situ (psychophysics assessment needed to be carried out before imaging because of time constraints for the imaging session), it required to be

connected and disconnected through the wave guide for each scan session. There were some initial practical issues with connecting the coolant pipes, which were overcome after some practice in setting up the equipment.

Thermode

A proprietary CHEPS (“Contact Heat Evoked Potentials”) thermode was employed to administer thermal stimuli to the study participant. The thermode incorporates a 27mm diameter ceramic disc, which is set up to be in contact with the participant’s skin (339). The temperature of the disc is controlled to within 0.5 degrees Centigrade by a user interface, employing a laptop running proprietary software. The manufacturer data states that the heating of the thermode disc is very rapid (70 degrees Centigrade/second), and that temperature is checked around 200 times per second, to ensure close temperature control (339).

Interface with study participant

In order to standardise site of administration, the Pathway device was attached to the medial right lower leg, 10 centimetres above the medial malleolus in every subject. The device was attached using proprietary Velcro strapping.

Psychophysical session prior to imaging

Assessments were carried out using a pre-prepared standardised written script. In all cases, the assessments were carried out by PF, in the same room and using the same equipment.

Prior to imaging, a short psychophysical session was carried out to establish two separate temperatures, for each research subject. This session was carried out in the mock scan room, adjacent to the MRI scan room, within the Clinical Research Imaging Centre (CRIC).

The first of these was a temperature which was reported as mildly painful (1/10 using a numerical rating scale, with the anchors of 0 being no pain, and 10 the worst pain imaginable). The second was a temperature which was reported as moderately painful (4-5/10 on the same scale). These temperatures were recorded individually for each subject. The temperature administered was adjustable within 0.5 degrees

Centigrade using proprietary software. These methods were comparable to previous studies of other pain syndromes, using similar equipment (340).

8.10.3 Scan session

Imaging acquisition

As described elsewhere in this thesis, a 3Tesla Siemens Verio system using a 12 channel head coil was employed. No scanner upgrades were carried out during the study.

Whole-brain EPI sequences were acquired (TR 2.5s, TE 30ms, 240 volumes, voxel size 3.0mm³) following acquisition of the structural, fieldmap and resting state fMRI data described elsewhere. Two separate scans of 10 minutes duration each were acquired (see below).

fMRI task

In the scan session, three second pulses of heat, at the predetermined temperatures (“Temp 1” and “Temp 2”) were administered with a jittered interstimulus interval of mean 60 seconds (range 55-65 seconds) (340). Presentation software (341) (set up by Cyril Pernet) was used to synchronize stimuli.

“Temp 1” was repeated 10 times, and in a separate EPI acquisition, “Temp 2” was repeated 10 times. For each subject, the order of Temp 1 and Temp2 was randomised so that half the cohort received Temp 1 then Temp 2, and the other half Temp 2 then Temp 1.

At the end of each 10 minute acquisition, the subject was asked for average pain rating (0 to 10, on the same scale) and also a rating of pain unpleasantness on a comparable numerical rating scale (0= not at all unpleasant, 10= most unpleasant imaginable).

Note: use of goggles during scan session

Initially, during exploratory work, the task fMRI scan session incorporated goggles, in order to allow visual presentation of stimuli to scan participants. Use of this equipment, however, in combination with the Pathway device described above, resulted in the introduction of extraneous radiofrequency (RF) noise during the scan acquisition. This was visible during imaging acquisition as a stripe through the brain at the level of the brainstem (an important region of interest in analyses of response

to painful stimuli). Subsequent imaging therefore did not use goggles. None of the data presented was acquired using goggles during the scan session.

Note: use of physiological noise modelling during session

Physiological noise monitoring was acquired during imaging acquisition (Cardiac and respiratory waveforms using a pulse oximeter and respiratory bellows, MP100, Linton Instruments, UK). Unfortunately there were several equipment and recording issues during scan sessions, and this data was not useable for physiological noise modelling in the subsequent analysis. Issues included fractured cabling, and problems with synchronisation with the MRI acquisition. There were also difficulties in some cases with maintaining adequate placement of the saturation probe, in particular. Because these issue were inconsistent and varied between and within acquisitions, this data was not used in subsequent analyses.

Analysis

Clinical variables

The following clinical variables were recorded

- Order of stimulation in fMRI (order of Temp 1 and Temp 2)
- Temperature 1 and Temperature 2 separately (degrees Centigrade).
- Pain score (numerical rating scale) for Temp 1 and Temp 2
- Unpleasantness score (numerical rating scale) for both Temp 1 and Temp 2
- Presence or otherwise of pre-existing neuropathic pain at stimulation site
- Pre-existing thermal allodynia at stimulation site

Normality of distribution of data was assessed using the Quantile-quantile plot.

Median and Interquartile Range were presented as summary statistics where data distribution did not follow Gaussian distribution. Statistical significance of differences between groups was assessed using the Wilcoxon rank sum test.

Statistical significance was accepted at the 5% level.

Task fMRI

Subject-level task fMRI was analysed by PF using FEAT version 6 packaged in FSL version 5.0.1 (105). The following analysis was carried out:

8.10.3.1.1 *Pre-statistics*

Motion correction using MCFLIRT (279) slice-timing correction using Fourier-space time-series phase-shifting; non-brain removal using BET (276); spatial smoothing using a Gaussian kernel of FWHM 5mm; grand-mean intensity normalisation of the entire 4D dataset by a single multiplicative factor; highpass temporal filtering (Gaussian-weighted least-squares straight line fitting, with $\sigma=45.0s$).

8.10.3.1.2 *Statistical analysis at the single-subject level*

Time-series statistical analysis was carried out using FILM with local autocorrelation correction. A double-gamma haemodynamic response function was used, in order to allow for undershoot in the BOLD response (71, 75). The time series was modelled in FEAT, using a temporal derivative (105).

8.10.3.1.3 *Registration*

Registration to high resolution structural and/or standard space images was carried out using FLIRT (279). B0 unwarping was used as described elsewhere in this thesis. Registration from high resolution structural to standard space was further refined using FNIRT (274).

8.10.3.1.4 *Higher level analysis*

A design matrix was constructed to calculate the contrasts of pain group BOLD activation > control group, as well as the opposite contrast, and mean BOLD activation within each group.

Higher level analysis was carried out using FMRIB Analysis of Mixed Effects (FLAME) version 1 implemented in FSL. Statistical significance was assigned at $z=2.3$ and corrected cluster forming threshold $p<0.05$ (342).

8.10.3.1.5 *Visual checking of results*

Brain extraction, B0 unwarping and registration were visually checked throughout the fMRI analysis procedure, using registration summaries produced by FSL and viewed in Firefox

8.11 Results

8.11.1 Recruitment and Patient data

45 subjects were included in the analysis (pain group n=29, control group n=16). Of the pain group, there was pre-existing pain at stimulation site (right lower limb) in 13. No subjects manifested thermal allodynia at the stimulation site.

23 of 39 underwent stimulation using “temperature 1” followed by “temperature 2” and 16 of 39 the converse.

8.11.2 Psychophysical assessment

For “temp 1” (pain severity 1/10 in the psychophysical session) median pain rating in the pain group was 2.0, and for the control group 1.0. Temperature applied in the pain group was higher in the pain group but this did not attain statistical significance.

For “temp 2” (pain severity 5/10 in the psychophysical session) median pain rating in the pain group was 4.0 and in the control group 3.0. Temperature applied in the pain group was higher in the pain group. Both of these differences attained statistical significance at the 5% level.

Table 52: Psychophysical data

	Whole group (n=45)	Control Group (n=16)	Pain Group (n=29)	
Temp (low)	44.00 (42.38-45.50)	43.5 (42.5 to 44.5)	44.5 (42.00 to 46.00)	P=0.2636
Pain (low)	2.00 (1.00 to 3.00)	1.00 (0.50 to 2.75)	2.00 (1.00 to 3.62)	P=0.0498
Temp (high)	45.75 (44.50-47.00)	44.50 (43.75 to 45.25)	46.00 (45.00 to 47.50)	P=0.01383

Pain (high)	3.50 (3.00-4.75)	3.00 (2.00 to 3.50)	4.00 (3.00 to 5.00)	P=0.01137
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Individual patient issues

Some individual participants found giving a reliable pain rating very problematic (see participant feedback for further description of this issue).

In some participants, reported pain due to stimulus in-scanner was markedly higher than reported pain due to stimulus pre-scan. For example, in one subject in particular (subject 0326) in-scanner pain was reported at 9/10, having previously been reported at 5 prior to imaging. Although this subject manifested high scores for anxiety and catastrophising (HADS A 14, PCS 25 (157, 233)) these were not the highest scores in the group. Therefore these issues in this particular patient were not easily predictable. This patient was a member of the pain group (ie had pre-existing neuropathic pain). These issues further increased the care that was taken to ensure that temperatures administered in-scanner were in a range acceptable to the participant.

8.11.3 Mean BOLD signal in pain group and control group

In both the pain group and control group, mean BOLD response included a variety of cortical and subcortical structures closely associated with pain response in previous studies (1). These included Insula, Somatosensory and Motor Cortices, Prefrontal Cortex, Anterior and Posterior Cingulate, Thalamus and brain stem. Please see the figures below.

Figure 47: Mean BOLD signal in control group, $n=16$. $Z=2.3$, $p=0.05$ (moderate pain severity stimulus: "Temp 2")

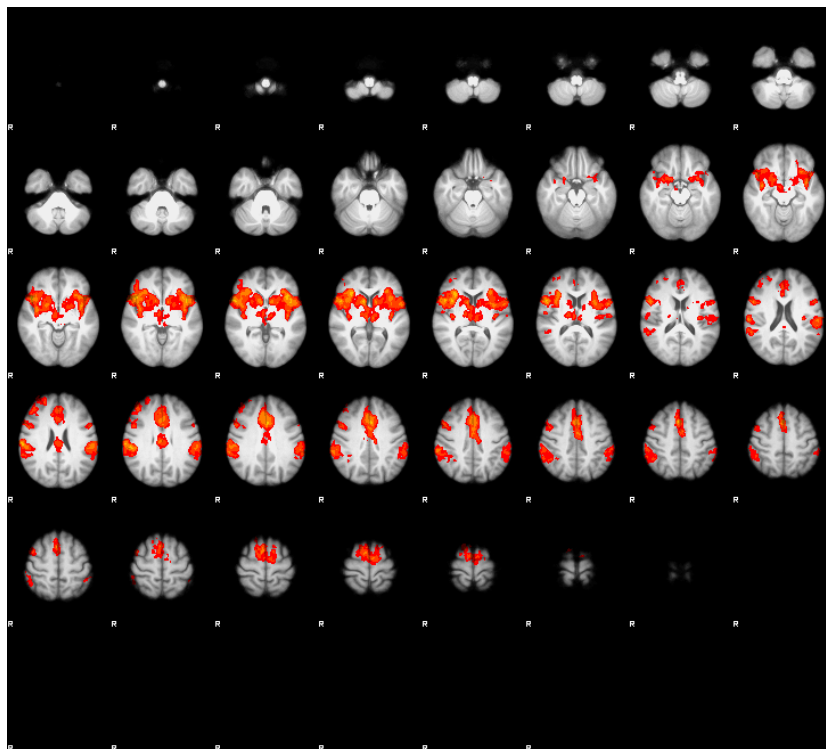
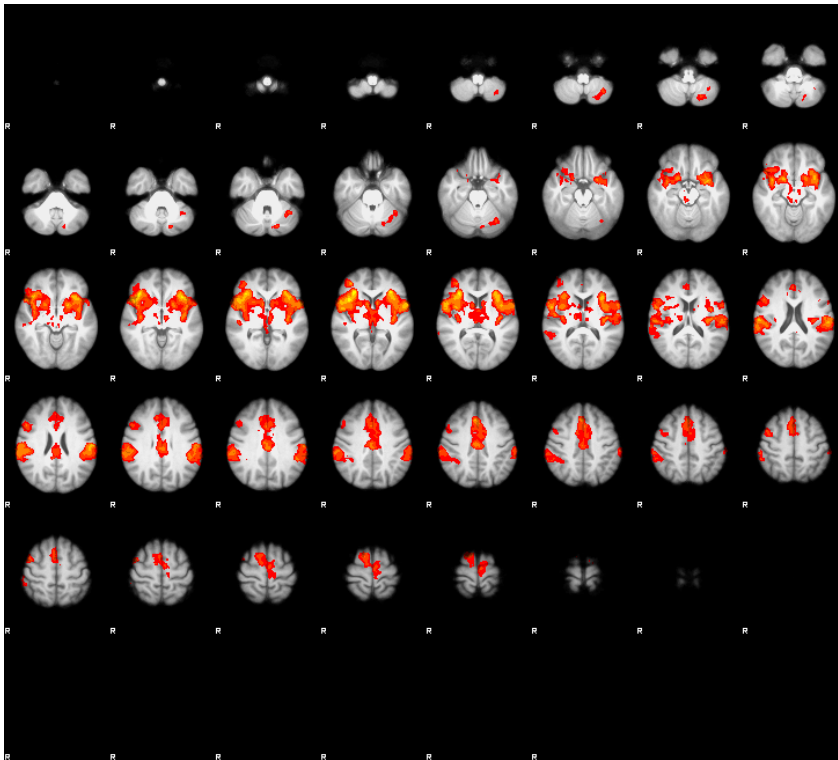


Figure 48: Mean BOLD signal in pain group, $n=29$. $Z=2.3$, $p=0.05$ (moderate pain severity stimulus: "Temp 2")



8.11.4 Statistical Contrasts between control and pain groups

In whole-brain contrasts between the two groups (5% significance level, and corrected for multiple comparisons) no statistically significant difference between the groups was found.

Further post-hoc subgroup comparisons contrasting those within the pain group who received the noxious stimulus at a site of pre-existing pain (the right lower limb) and those who did not have pre-existing pain at that site, did not reveal any statistically significant difference between groups.

Similarly, a contrast between participants in the pain group who received, and who did not receive, adjuvant analgesics did not demonstrate any statistically significant difference between groups.

8.12 Discussion

This study is presented as a work in progress, as part of the overall thesis. Specific strengths and weaknesses, and potential relevance to methods and data described elsewhere in this thesis, are discussed.

8.12.1 Weaknesses

Variability in pain scores

Pain scores generated immediately prior to the imaging session (in all cases) proved to be only moderately useful in estimating pain severity during noxious stimulation in-scanner. This introduced some difficulty in interpretation, which was addressed by recording both temperatures and pain scores for each scan session in each subject.

Stimulus-correlated motion

Stimulus-correlated motion was a potential issue in the interpretation of this data. This concept refers to subject head motion which is correlated to stimulus presentation during an fMRI scan session. This may particularly be marked in “pathological” populations such as people with multiple sclerosis, and/or with chronic pain. The interaction between chronic pain and presence of MS in the context of subject motion in a noxious stimulation fMRI study is unknown (75, 166). Removal (“scrubbing” or “censoring”) of high-motion timepoints however risks removal of the BOLD signal of interest (75, 102).

Average pain ratings across ten trials

Pain ratings were averaged over 10 consecutive stimuli. All though the 10 consecutive stimuli were identical, trial-by-trial ratings were not possible. This issue relates in particular to technical problems in use of the goggles during fMRI acquisition (as these would have allowed trial-by-trial ratings).

8.12.2 Strengths

Novelty

This study represents, to the best of the author’s knowledge, the first use of task based fMRI paradigms of any type, to investigate the mechanisms of neuropathic

limb pain in MS. The study is also the first to use noxious thermal stimuli in an fMRI paradigm to investigate the mechanisms of neuropathic limb pain in MS. Although unpublished data may exist, systematic review of existing data supports these conclusions (166).

Nature of stimulus

The temperature of the noxious stimulus, while it can be calibrated for each participant's response, can be measured to the nearest 0.5 degrees Centigrade (according to manufacturer data (339)). Administration of the stimulus, once the equipment is set up, is entirely computer-controlled, with subsequent limitation in variability in the stimuli during the imaging session. This might lead to less variability in the stimulus than might be expected for, for instance, a manually-controlled stimulus.

The nature of the thermal noxious stimulus is designed to qualitatively mimic the nature of neuropathic limb pain in MS, which is often related to have a "burning" quality (5, 69, 144).

A jittered inter-stimulus interval was used to minimise participants' ability to correctly anticipate the administration of the noxious stimulus (75).

8.12.3 Perspectives in relation to resting state analysis

The use of a task fMRI paradigm in addition to a resting state fMRI paradigm may from some perspectives be viewed as complementary. A resting state paradigm allows exploration of the brain's functional connectivity "at rest" (which may be viewed as closer to the participant's own experience of the situation explored, in this instance neuropathic limb pain, at least in as much as the condition of lying in an MRI scanner may mimic real life). A task fMRI paradigm is however useful to explore BOLD signal associated with extrinsic stimuli. While these may not directly mimic the participant's own experience of pain, the high degree of control a researcher exerts over the stimulus allows detailed examination of the BOLD signal associated with administration of the stimulus (75, 340).

The use of an extrinsic stimulus can be criticised in that, while it allows investigation of specific physiology underlying the participant's pain experience, it is necessarily different from the subject's own pain experience. It may therefore introduce interpretation difficulties, particularly where used in isolation, and other methods such as resting state fMRI or ASL (Arterial Spin Labelling) may be preferred from this perspective (343).

8.12.4 Conclusions

The data presented here may be useful as the subject of future analysis.

In particular, this data could be used to explore specific hypotheses regarding the functional integrity of the descending pain modulatory system, suggested by the analysis of the resting state data. Techniques developed for the analysis of functional connectivity in task-based fMRI paradigms, for instance psychophysical interaction (PPI) and dynamic causal modelling (DCM) might be particularly useful in this regard (344, 345).

Because of the methodological issues outlined above, particular care will need to be taken in such analyses, to ensure that results are readily interpretable. Such measures could include appropriate correction for confounders (covariates of no interest), or specified subgroup analyses.

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